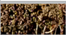
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# Soil Microbiology and Biochemistry

Third Edition



Edited by Eldor A. Paul



# Soil Microbiology, Ecology, and Biochemistry

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Third Edition

Editor
Eldor A. Paul

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J. Schimel

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# PREFACE

Soil microbiology traditionally has been the study of microorganisms and their processes in soil. The interaction of organisms with each other and their environments involves soil ecology. Soil biochemistry includes microbial processes, soil enzymes, and the formation and turnover of soil organic matter. Soil, in the nonengineering definition, is usually defined as the surface of the earth affected by plant roots, even though life, especially that of microorganisms, occurs at great depths in geological deposits, caves, and sediments. Although the organisms involved are often different, their ecological and abiotic controls and the products of their metabolism have great similarities in all locations. Thus, there is now a recognized similarity and interaction with soil and biogeochemical studies in marine and fresh water systems, sediments, and the atmosphere. What we know from these processes on earth will also guide future extraterrestrial investigations and, as a result, the number of people interested in this field has greatly increased. The textbook "Soil Microbiology and Biochemistry" by Paul and Clark (1989, 1996) is available in Chinese and Korean translations. It has been incorporated into the teaching of engineering, biogeochemistry, ecology, and general biology in a variety of university departments, including those of private, undergraduate, and teaching universities, and is widely used in many research applications.

The biological processes that occur in soil are intertwined with and inseparable from activities of the soil fauna, which feed on plants, soil microorganisms, and litter. Their larger forms act as environmental engineers through their soil-mixing functions. They also contain microbial endophytes that carry out much of their decomposition function. The name of this edited volume has been changed to reflect its broader applicability and has been expanded to include both more basic and applied approaches. Soil microbiology, ecology, and biochemistry are being

used in a broad range of applications from agronomy, plant pathology, general ecology, microbial ecology, engineering, organic agriculture, forestry, range management, and global change. We have thus included chapters on invertebrate–microbial interactions, basic physiology, and ecological interpretations. Information on the management of microorganisms and their reactions has been expanded while we have strived to retain readability, conciseness, and a reasonable cost.

The definition of microbiology is usually associated with organisms not seen without the use of a microscope, although this does not apply to many fungal lichen and algal growth forms. The communal structure of the *Armillaria* associated with tree roots in a number of areas is hectares in size, although it is still a fungus by definition. The soil fauna also range in size and diversity. This book reflects the great advances in molecular techniques, the broader use of tracers, and the maturation of modeling in interpretation of data and development of new concepts. We finally know enough about our field to be able to impact management of such modern problems as biodiversity, biological invasions, global change, ecosystem services, sustainable agriculture, and urban ecosystems. This textbook has been designed to provide access to necessary knowledge for those working in these diverse fields. The authors of the individual chapters hope that the readers will find this a readable, accessible introduction to both the concepts involved and the background literature.

PART

# I

# Background

# 1

# Soil Microbiology, Ecology, and Biochemistry in Perspective

E. A. Paul



## GENERAL HISTORY AND SCOPE

The processes that occur within soil are closely related to those in sediments and aquatic environments. They are also associated with the beginning of life on this planet. Biochemical and biological changes were associated in the earth's early stages. Molecular biomarkers, isotope modification (such as differences in $^{34}S$ and $^{13}C$), and identifiable fossils are important in the study of the earth's history. The primordial soup theory of Oparin and Haldane assumed that organic compounds in water underwent polymerization and condensation reactions similar to those that describe modern soil organic matter formation. The formation of macromolecules that catalyze their own replication is known to be assisted by clays, metals, imidazole derivatives, and selective adsorption onto mineral surfaces that promote concentration and polymerization (Bada and Lazcano, 2003). Carbon and associated N substrates may have arrived on meteorites in association with minerals.

The first written history of soil and soil biota originated in the East, where scholars were recognized in the early Chinese royal courts. Coleman *et al.* (2004)

stated that soils were classified during the Yao Chinese dynasty from 2357 to 2261 BCE. This dynasty should be recognized for both basic and applied studies of soils as they used a soil classification for taxation purposes. The ancient Chinese regarded earthworms "as angels of the earth." Romans, such as Aristotle, considered earthworms as "intestines of the earth" (Coleman *et al.*, 2004). Further evidence for the early recognition of soil is that the Hebrew word for soil is "adama," from which is derived Adam, the first man in Semitic religions (see Hillel, 1991). The ancient Vedic literature of India classified soils by color (and thus organic matter content) and recognized the importance of land forms, erosion, vegetation, land use, and human health implications.

Fungi were known for their fermentation reactions in wine, beer, and bread making and also as a food source that could at times be toxic. Inscriptions on Egyptian walls from 2400 BCE show the production of beer and bread involved the use of a starter and required an incubation time. Eastern, and later Roman, scholars recognized the soil-improving qualities of legumes and crop residue additions. Roman literature on agriculture and soil management was extensive. This was updated and condensed into a single volume by Petrus Crescentius in 1240 CE and for many years was copied, even into the time of the printing press (e.g., Ruralium Commodorium libri duodecin Augsburg, 1471).

Knowledge stagnated in Europe for the one and a half thousand years prior to the Renaissance at the end of the 15th century; not from a lack of intelligence, but from the firmly held belief that the world was governed from the outside and was not an object to be questioned (i.e., intelligent design). The end of the 15th century marked the end of the Western medieval world with the emergence of the perspective that laws that govern the world are subject to study. The concept of biological and abiotic controls that can be studied and influenced by humans marked the beginning of our present knowledge of the soil biota and their processes. The ability to transmit this knowledge by the printed word after the invention of the printing press also greatly aided scientific discovery and discussion.

We are getting further away from our historical roots, an understanding of which is so important to our thinking and ability to formulate scientific questions. The advent of the computer with its easy access to recent literature seems to delay visits to the library to look at not only the original thinking in our field during the early 20th century, but also important literature from 1950 to 1980. I have tried to summarize briefly some of the important early discoveries. In doing so, I have not referred to the original literature, but to reviews often found in textbooks that should be available in many libraries. The history of our science is not merely a listing of the important discoveries, but an important example of scientific thought processes and the relation between methodology, ideas, and concepts.

Our field is still methodology-driven as shown by the great increase in knowledge being derived from molecular techniques and tracers. Another methodology breakthrough was nearly driven to excess, as shown by the fact that the three most cited papers from the *Soil Biology and Biochemistry Journal* from 1975 to 2000

involved the application of the fumigation technique (earlier used by Schloesing and Müntz for nitrification studies) for the measurement of microbial biomass. Today we are benefiting greatly from the availability of automated techniques, the use of computers in data transformation, modeling and knowledge dissemination, and the presence of active scientists in many new parts of the globe.

A look at our history shows how ideas were generated. It also shows that we should look at some of the misconceptions of the past to help us clearly define our thoughts and concepts. I realize that my biases show and that I have concentrated on the positive. The literature is full of examples showing that many of our founders also developed some "doozies." It would also be rewarding to look at what did not pass the test of time so that our own ideas do not end up in the same dustbin. A brief survey of citations in some search engines, such as the U.S. National Agricultural Library, Commonwealth Agricultural Bureau, ISI Science Citations, and Biological Abstracts, shows that the words "soil ecology" elicit more responses than "soil microbiology," which is followed in interest by "soil biochemistry" and "microbial ecology." There are differences in relative rankings dependent on the search engines, but processes generally involve more citations than microorganisms. Soil N is most popular, followed by soil C, $N_2$ fixation, and the rhizosphere. The citation survey shows that new methods of analysis are being applied to continuing problems with pollutants and pesticides and their effects on the soil population. These topics are continuing to receive a great deal of attention, as is soil biodegradation. If you really want to gain a further appreciation of our field, try general search engines, such as Google, which lists 9,050,000 items for "soil microbiology," 25,100,000 for "soil ecology," and 7,800,000 for "soil biochemistry." An understanding of the interest in the word "humus" would require the perusal of 4,760,000 items. This, however, includes recipes for a common Mediterranean prepared food, hummus, so maybe a better search would be for "soil organic matter," with 14,600,000 items.

## SOIL MICROBIOLOGY

Fungi in certain forms can be readily seen without a microscope; thus, they received early study. The first book solely about fungi ("Theatrum Fungorium") published in 1675 by J. F. van Starbeck drew heavily on the drawings of Charles de'Egeluse prepared as early as 1601 (see Atlas, 1984). In 1665, Hooke published a work on the fruiting bodies of fungi, and by 1724, spores were known as fungal reproductive agents. Fungus–root associations were noted by earlier authors, but in 1877, Pfeffer recognized their symbiotic nature, and in 1885, Franck coined the word "mycorrhiza." Franck later distinguished between ecto and endo associations; a classification that is still applicable in present, extensive literature on this subject. In 1886, Adametz isolated fungi from soil and gave them names. The first detailed classification of soil fungi was conducted by Oedemans and Koning in 1902 (see Waksman, 1932). In the 1920s, Charles Thom made a detailed study of

soil fungi, especially *Penicillium* and *Aspergillus*, the dominant soil fungi on most agar plates. Waksman also published extensively on soil fungi and actinomycetes.

Leeuwenhoek (1632–1723) is recognized as being the first to see bacteria in his self-designed microscopes. He observed the small animalcules in natural water and in water amended with a substrate (pepper or meat broth). The comprehensive classification system produced by Linnaeus in 1743 perhaps foretold the modern difficulties in bacterial classification when he placed all the organisms seen by Leeuwenhoek in infusions of vegetable matter and meat broth into the genus *Chaos.* In 1776, Nagelli (see Atlas, 1984) suggested that bacteria be placed into their own class entitled Schizomycetes. The work of Warington, Lawes, and Gilbert established the biological nature of many of the processes involved in N transformations, especially those involved with the growth of leguminous crops. Pasteur (1830–1890), in discrediting the theory of spontaneous generation, laid the foundation for microbiology. Although trained as a chemist, he developed vaccines for rabies and investigated many food microbiology problems. Pasteur and Liebig had both postulated that the process of nitrification was bacterial in nature. While studying sewage purification by land filters, Schloesing and Müntz found that the ammonia content of sewage passed through a sand filter did not alter for 20 days. After this period, ammonia was changed to nitrate, but the process could be stopped by a small amount of chloroform. The process could be restarted by soil extract, thus proving that this process was due to microorganisms or, as they said, "organized ferments."

S. Winogradsky (1856–1953) is recognized as the founder of soil microbiology for his contributions to nitrification, anaerobic $N_2$ fixation, sulfur oxidation, and microbial autotrophy (Winogradsky, 1949). He succeeded in isolating two bacterial types involved in nitrification with the keen insight that they obtained their C from $CO_2$. He thus also established autotrophy in microorganisms. In the period 1872–1876, Cohn published the first comprehensive study of the bacterial content of soil. Hellriegel and Wilfarth, in 1888, grew peas in the absence of a fixed N supply, showing that legumes obtained their N from the atmosphere, whereas oats did not have this capability. They knew that the peas had nodules, but could not isolate the bacteria within. Beijerinck, in 1888, isolated the bacteria that he called "*Bacillus radicicola*" (now usually called "*Rhizobium*"). This showed the dependence of the N cycle on bacteria. The N cycle was completed when Goppelsröder observed that nitrates were reduced to nitrites in the presence of soil organic matter. In 1868, Schoenbein ascribed the reaction to bacteria and Gayon and Dupetit further developed the knowledge that led to denitrification studies.

The latter half of the 19th century saw more details on microbial processes including symbiotic and asymbiotic $N_2$ fixation, denitrification, and sulfate reduction and oxidation. The research on fermentation led to the delineation of anaerobic metabolism. Waksman, in his 1952 textbook "Soil Microbiology," gives a detailed account of the early contributions and also published photographs of many of our academic forefathers in soil microbiology. His 1932 book gives detailed historical references in each of the chapters, as well as a listing of the textbooks on the various topics to that date. He gives credit (together with Winogradsky) for the foundation of soil

microbiology as a discipline to Martinis Beijerinck (1851–1931), who not only extracted the first viruses from plants, but also isolated many $N_2$-fixing organisms and developed enrichment techniques. Basic and applied sciences were as intertwined in the beginning of our science as they are now. Winogradsky and Beijerinck are also recognized as founding members of microbial physiology and microbial ecology.

The first textbook to include soil microbiology was that of Löhnis, "Vorlesunen über Landwirtschaftliche Bäkteriologi," published in 1910 and 1913. English readers can gain an insight into its contents in the English version he published together with E. B. Fred in 1923, entitled "Textbook of Agricultural Bacteriology." That text contains very readable accounts of bacteria, fungi, and protozoa and a good discussion of relationships of microorganisms to their environment. J. G. Lipman (1874–1939), who established the Department of Soil Chemistry and Bacteriology at Rutgers University in 1901, was especially interested in the effects of soil organisms on soil fertility and plant growth. His 1911 book entitled "Bacteria in Relation to Country Life" was the first American treatise in this field. Waksman (1952) named the period from 1890 to 1910 as the Golden Age of soil microbiology when representatives of the soil biota carrying out the major soil and biogeochemical processes were identified. The identification of at least representative members of the microorganisms mediating soil fertility and nutrient transformations led to the belief that this knowledge could do for agriculture what the identification of major disease organisms did for medical treatment.

Successes in legume inoculation led to several premature attempts to alter soil C and N transformations by inoculation and to relate microbial numbers to soil fertility. This discussion continues to this day in the many questions concerning biodiversity and ecosystem functioning addressed later in this volume. The attempts to inoculate bacteria, other than symbionts, and control microbial pathogens of plants were seldom successful because of the lack of knowledge of microbial ecology and the other controls involved. These studies did, however, help transfer attention from pure cultures and laboratory investigations to field experiments and the need for replication to account for soil heterogeneity. This period also contained the interesting conclusion that if an organism did not grow on a gelatin or agar plate, it could not be important and thus was not worth studying.

The years from 1910 to the Second World War witnessed the employment of soil microbiologists in numerous new institutions in many parts of the world. This led to a better knowledge of the global distribution of, and management effects on, organisms capable of growth in the laboratory medium. The development and use of direct microscopy led to the realization that approximately only 1% of the soil population could be grown on laboratory media. The failure of inoculants, except in the case of symbiotic $N_2$ fixation, to create meaningful management effects was a worry at that time. It is only now that we realize the huge number of unidentified organisms and that the unknown interactions between them and their environment (ecology) explain the often observed lack of impact of introduced organisms.

It was at first assumed that bacteria were the major players in soil fertility and decomposition as typified by the books of Löhnis in 1910 and Löhnis and Fred in

1923. In 1886, Adametz showed that fungi are abundant in soil. Additionally, Hiltner and Störmer had studied actinomycetes, which at that time were thought to be different from the bacteria. Cutler had studied the protozoa, and Russell and Hutchinson developed the theory that by consuming bacteria, protozoa could control the soil population and, thus, soil fertility. The early textbooks took as much license with their titles as modern ones. The Löhnis and Fred publication on agricultural bacteriology included extensive sections on the protozoa and fungi discussed under sections such as "Bacteria and related microorganisms." Waksman's "Soil Microbiology" included sections we would today call biochemistry. The effects of environmental factors on the rate of soil organic matter decomposition were described by Waksman in his 1932 book entitled "Principles of Soil Microbiology" and the Waksman and Starkey 1931 book entitled "The Soil and the Microbe."

The period between the two world wars saw work on microbial interactions and nutrient transformations. Fred, Baldwin, and McCoy's 1932 comprehensive volume on "Root Nodule Bacteria and Leguminous Plants" set the stage for the continued success in symbiotic $N_2$ fixation. The C:N ratio required for plant-residue decomposition without N immobilization was determined as approximately 25:1, a number that is still appropriate unless large amounts of poorly degradable residues are involved, as in forest litter. Attempts to measure many of the microbial processes in soil were frustrated by the inaccuracy of the measurement techniques relative to the large stock of nutrients in soil. Waksman (1932) commented that it was difficult to measure $N_2$ fixation by free-living organisms at levels less than 40 lb per acre, which was (and still is) the inherent error in the Kjeldahl or other methods of measuring total N. The Finnish scientist A. I. Virtanen received the 1945 Nobel Prize in Chemistry for his major contributions to legume nutrition, especially the role of rhizobia in symbiotic $N_2$ fixation. Lie and Mulder (1971), in "Biological Nitrogen Fixation in Natural and Agricultural Habitats," provide a record of the many advances made in that field.

The Second World War led to a concentration on the war effort. This was, however, not without its success as witnessed by the use of the fungal antibiotic, penicillin, and the development of streptomycin, for which Waksman received the Nobel Prize in Medicine in 1952. The war also resulted in studies to overcome food spoilage and rotting of clothes, as well as the beginnings of biological warfare in both preventive and causative formats. Alexander's 1961 and 1977 "Introduction to Soil Microbiology" continued the general organization utilized by Waksman in his earlier volumes. He organized the section on the soil environment and bacteria, actinomycetes, fungi, algae, protozoa, and viruses into a section entitled "Microbial Ecology" and recognized the multitude of microbial and microbial–plant interactions. The 1960s saw an influx of new scientists that worked on symbiotic and asymbiotic $N_2$ fixation, S cycling, the rhizosphere, mycorrhizas, and the effects of herbicides, pesticides, and pollutants on the microbial population. The mycorrhizal history to 1969 can be found in Harley (1969). The use of $^{15}N$ and alternate substrates and inhibitors for specific enzyme interactions made possible for the first time the quantification of the processes in the N cycle at the levels that they occur in soil. However, method availability still hindered testing of concepts regarding

microbial populations and diversity, and it was not until the advent of nucleic acid methodology, automated biochemical measurements, such as phospholipid fatty analysis (PLFA), computers, and modeling that the great thrust of knowledge covered in the subsequent chapters of this volume could come to fruition.

Volumes on soil microbiology include Subba Rao (1999), “Soil Organisms and Plant Growth,” 4th ed.; Killham (1994), “Soil Ecology;” Lynch (1983), “Soil Biotechnology;” Metting *et al.* (1992), “Soil Microbial Ecology;” Alef and Nannipieri (1995), “Methods in Applied Soil Microbiology and Biochemistry;” Van Elsas *et al.* (1997), “Modern Soil Microbiology;” and Sylvia *et al.* (2005), “Principles and Applications of Soil Microbiology.” Other volumes include Tate in 1994, “Soil Microbiology;” Harley and Smith in 1983, “Mycorrhizal Symbiosis;” Read *et al.* in 1992, “Mycorrhizas in Ecosystems;” and Makerji, Chamola, and Singh in 2000, “Mycorrhizal Biology.” A community and ecosystem approach to the biology of soil is presented by Bardgett (2005) and the role of microbial diversity as a supplier of ecosystem services is presented in two edited volumes (Bardgett *et al.*, 2005; Wall, 2004).

The advances in molecular techniques utilizing culture-independent direct retrieval of 16S rRNA genes have allowed an examination of the occurrence and biodiversity of microorganisms. A survey conducted by Morris *et al.* (2002) examined the primary scientific literature from 1975 to 1999 in 525 journals. Figure 1.1 shows data for six soil-associated habitats.

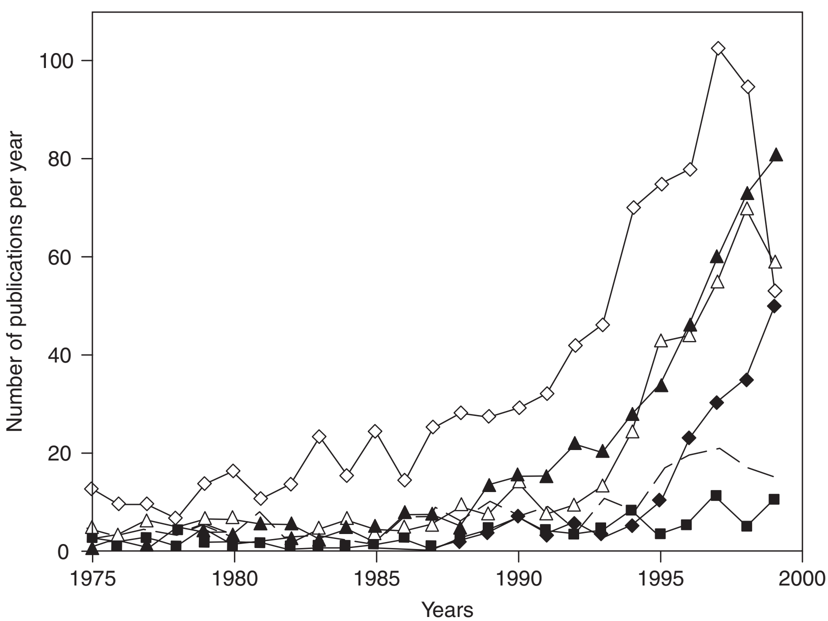



FIGURE 1.1 Publications per year from 1975 to 1999 in microbial diversity: (◇) fungal–plant pathosystems, (▲) rhizosphere and mycorrhiza, (△) microbial habitats in soil, (◆) aquatic systems, (—) bacterium plant systems, and (■) food microbiology (Morris *et al.*, 2002).

Fungus–plant pathosystems outnumbered the other five habitats and showed a 10-fold increase in papers; however, that number peaked in 1996. The rhizosphere, including mycorrhizas, was still rapidly increasing in popularity in 1999. Microbial habitats in soil showed a similar trend, as did aquatic systems. Molecular techniques hold great promise for increasing our understanding of the links between organisms, processes, and the environment; thus soil microbiology, biochemistry, and ecology are best treated in one volume. The recent finding of ammonia-oxidizer genes in previously immeasurable Archaea is one example of new functional groups and maybe even new functions and processes that will be discovered by the readers of this book.

## SOIL ECOLOGY

Soil ecology is the second leg of the scientific tripod supporting this textbook. Ecology has numerous definitions. The one that applies to this text is the interaction of organisms and their environment. Smith and Smith (2001) stated that Haeckel developed the term "ecology" in 1869 from the Greek term "oikos," meaning home or place to live. The first ecological publications are credited to the Greek scholar Theophrastus (371–288 BCE), who wrote nine books on "The History of Plants" and six on "The Causes of Plants." Continued work by naturalists during the 15th century, especially in the Middle East, was followed by the plant geographers, such as Wildenow (1765–1812) and Von Humboldt (1769–1859). These described vegetation by physical type and environmental conditions and coined the word "association" (see Smith and Smith, 2001). More plant geography, such as that of Schouw, who studied the effects of temperature on plant distribution, and Paczoski, who studied microenvironments created by plants, led to the study of plant communities. Scientists such as Coulter, Bessey, and Clements developed concepts of succession and gave ecology its hierarchical framework (see Major, 1969).

Aquatic research contributed much to ecological theory. In 1887, Forbes, who interestingly had no college degree (see Hagen, 1992), wrote the classic "The Lake as a Microcosm," which was a predecessor to ecosystem ecology and introduced the concepts of interrelationships through food chains. In 1931, European biologists Thieneman and Forel used the concept of organic nutrient cycling and developed the terms "producers" and "consumers." In 1926, agronomist Transeau was interested in improving agricultural production through a better understanding of photosynthetic efficiency and initiated our understanding of primary production. The early ecologists tended to concentrate on native plant and animal associations, whereas at that time soil microbiologists were associated with either agronomy or microbiology departments. Agronomists were primarily concerned with cultivated fields and the processes therein. To the soil zoologists, these fields seemed depauperate of interesting organisms, while the ecologist's obsession with native sites, and to some extent the environmental movement, was thought by the agronomists to greatly limit their interpretive capability.

Ecosystem science, a term coined by Tansley in 1935 (see Hagen, 1992), led to a more experimental approach and interdisciplinary work. The textbook organized around the ecosystem concept, "Fundamentals of Ecology" by E. P. Odum (1971), went through three editions and was translated into more than 20 languages. The International Biological Programme of the 1960s and 1970s demonstrated the need to investigate all the interacting components of the ecosystem and to model them using mathematically defined transformation processes. This required the active interaction of soil microbiologists and biochemists with plant and animal ecologists and agronomists. During this program, G. M. Van Dyne, a strong advocate of the ecosystem concept, described the editor of this volume as standing on a four-stranded barbed wire fence between ecology and agronomy, with the warning that some day I would slip, with the obvious drastic consequences. The title and chapters in this book indicate to me that this fence has finally been ripped out. Future great advances lie in the study of our exciting field by scientists with a variety of backgrounds and employment in institutions often as heterogeneous as the soils and organisms they study. At the same time, the more classically trained ecologists recognize that the soil, with its multitude of interacting organisms and complexity of interactions, is the last great frontier of ecology.

Today's researchers are finding that replicated, managed fields are excellent for studying and developing ecological and biogeochemical concepts in that they often have greater, more easily measured, nutrient fluxes than those in perennial vegetation. Uncultivated systems, whether prairie or forest, are essential as reference points, often with greater diversity. Other work, such as that in the Amazon Basin, is recognizing that many of the forests that were once thought to be pristine have had major past human interventions.

Russell's 11th edition of "Soil Conditions and Plant Growth," edited by Wild (1988), noted that Gilbert White, in 1777, observed that earthworms were promoters of vegetation by perforating and loosening the soil and drawing leaves underground. Feller *et al.* (2003a) note that Darwin first reported on the effect of earthworms in 1837, followed 34 years later by the publication "The Formation of Vegetable Mould through the Action of Earthworms." At that time, the term "vegetable mould" was used to designate surface horizons in a manner not that different from the earlier use of the term humus. Darwin showed that earthworms were important in soil formation by affecting rock weathering, humus formation, and profile differentiation. This led Feller *et al.* (2003a) to credit Darwin for the first scientific publication in Europe on the biological functioning of soils. In 1839, Ehrenberg had shown the presence of soil protozoa (see Feller *et al.*, 2003a). Russell's work on partial sterilization and its benefits to fertility had involved the protozoa. Cutler and Crump, in 1920, observed the often reciprocal increase and decrease of amoebae and bacteria and attributed the concept of soil sickness resulting in lowered fertility to this phenomenon (see Waksman, 1932). This is in direct contrast to Russell's, and more recent, concepts in which faunal-derived microbial turnover is considered an advantage in nutrient release (Coleman *et al.*, 2004). Stout *et al.* (1982) gave a detailed resume of the soil protozoa that included the slime molds.

The "Manual of Agricultural Helminthology" (Filipjev and Shuurmans-Stekhoven, 1941, published in The Netherlands), summarized nematode anatomy, systematics, methodology, and plant–parasite interactions to that date. G. Steiner states in the edited volume on nematology (Sasser and Jenkins, 1960) that the Incas of Peru had a regulation by which the replanting of potatoes on the same land needed to be deferred by a few years to control what must have been golden nematode infestation. He also stated that the "bush culture" that involved burning of tropical forests followed by planting of crops was not done on adjacent plots to stop invasion of nematodes from the old agricultural plots to the new ones. Kevan's 1965 description and count of soil fauna per square meter of a European grassland were quoted in the first edition of this textbook. A good introduction to the various members of the soil fauna is given by Burges and Raw (1967) and is updated by Lavelle and Spain (2001) and Coleman *et al.* (2004).

Wilde (1946) stated that the principals of soil science and ecology were introduced to silviculture by the German forester Grebe in his doctor's thesis in 1840. Grebe forecast Dokuchaiev's studies by stating,

> "As silviculture horizons widen, the importance of environmental conditions becomes more sharply pronounced. It appears clearly to foresters that the form of forest management is determined by a number of physical influences related to topography, geology, type of soil, and climate."

In not mentioning organisms, maybe the quote does not belong in this book, but 80% correct isn't all bad.

Russian scientists have long credited Dokuchaiev and his associate Kostytchev with being the founders of soil science and for having a great influence on ecology. Wilde (1946) quotes Dokuchaiev as saying,

> "The eternal genetical relationships that exist between the forces of the environment and physical matter, living and nonliving domains, plants and animals and man, his habits, and even his psychology—these relationships comprise the very nucleus of natural science."

Dokuchaiev recognized the effects of animals in soil formation in using the word "crotovina" for the filled-in remnants of mammal burrows. Russian soil science, ecology, geography, and plant ecology have always been closely associated (Major, 1969). Their word "biogeocoenoses" emphasizes the biology–landscape interactions, as well as exchanges of matter and energy, discussed so often in this text. Hilgard translated Dokuchaiev's work to English and mapped American soils relative to landscape, climate, and vegetation. Wilde credits Hilgard's 1906 publication "The Relation of Soils to Climate" for perhaps unintentionally laying the foundation of soil ecology in America. The interactions of Dokuchaiev's five factors of soil formation, climate, parent material, organisms, topography, and time were reiterated and placed in an equation form by Jenny (1941). Liebig has been credited as one of the first physiological ecologists for his work on mineral nutrition of plants.

The influence of Müller's 1878 monograph in characterizing forest soils in relation to the type of organic matter (Mull, Moder, and Mor) has been extensive. Wilde lists an extensive number of European authors who emphasized the role of soils in forest management. Other reviews on forest–microbiology–nutrient cycling include Jordan (1985), Pregitzer (2003), and Morris and Paul (2003). Rangeland science is equally dependent on soil processes, some of which are detailed in "Grasslands, Systems Analysis and Man," edited by Breymeyer and Van Dyne (1980), and in "Grassland Ecophysiology and Grazing Ecology" (Lemaire *et al.*, 2000).

I did not know whether to place microbial ecology under soil microbiology or soil ecology. In concepts, methods, and application, microbial ecology has been closer to soil microbiology than to classical ecology. Numerous authors have bemoaned the fact that there is not an extensive idea and concept exchange between microbial ecology and ecology in general. However, this is rapidly changing with the recognition that the diverse and extensive soil and aquatic and sediment biota can now be studied with molecular methods. The great diversity and close interactions of organisms with mineral particles makes soil an ideal place to develop and test ecological concepts. According to Marshal (1993), microbial ecology has the goals of defining population dynamics in microbial communities and the physiochemical characteristics of microenvironments and understanding the metabolic processes carried out by microorganisms in nature. It recognizes as its founders the same scientists (Leeuwenhoek, Winogradsky, and Beijerinck) that developed soil microbiological thought. Microbial ecology has the ability to transcend different habitats, asking questions about soils, plants, animals, fresh waters, oceans, and sediments, as well as geological strata. It also has received great impetus from the recent advances in nucleic acid techniques and, thus, one of its more modern pioneering works has to be that of Watson and Crick, which eventually led to the nuclear-based techniques.

The first textbook published with the title "Microbial Ecology" was that of Brock (1966). Brock (1975), in "Milestones in Microbiology," published the key papers of Pasteur, Koch, and others in a translated, annotated format. The publication of the triennial meetings of the International Society of Microbial Ecology provides a useful chronology of advances in this field. Some include Ellwood *et al.* (1980), "Contemporary Microbial Ecology;" Klug and Reddy (1984), "Current Perspectives in Microbial Ecology;" and Guerrero and Pedros-Alio (1993), "Trends in Microbial Ecology." Other reviews include Lynch and Poole (1979) and the series "Advances in Microbial Ecology" published by Plenum Press. The training and background of microbial ecologists are often very different from those of classical ecologists, and until recently, there has not been enough cross-fertilization of ideas between the fields.

## SOIL BIOCHEMISTRY

Soil biochemistry, as defined in this book, refers to the characteristics and dynamics of organic matter and the biochemical transformations brought about by

enzymes and organisms in soil. Biochemical reactions appear to have proceeded without microorganisms. Later microorganisms were active without the presence of plants and animals for long periods of the earth's history. Biochemical reactions similar to those occurring in modern soils are thought to have occurred for an extended period before the occurrence of the first bacteria identified in rocks that have an age of approximately 3.8 billion years. Phototrophic bacteria and cyanobacteria have been identified in rocks that are 2.8 billion years old. Vascular plants and mammals are a product of only the past 500 million years.

Experiments with iron sulfides, at the elevated temperatures and pressures found in hydrothermal vents, have indicated the possibility of the formation of prebiotic, organic substrates. These are believed to involve organo-metal interactions often studied in today's soil biochemistry. Another theory involves an alkaline world in which the activity of negatively charged clay minerals, such as smectite, organized fatty acid micelles and lipids into vesicles that contained active clays. These are said to have concentrated and polymerized RNA and DNA. Once formed, vesicles such as these are postulated to have grown by extrusion through small pores. These reactions are all familiar to the soil biochemist, as are the concepts involving micropores, enzymatic activity, and habitat formation so important in early life studies (Bada and Laszano, 2003).

Waksman (1938), in his book entitled "Humus," states that from Theophrastus (373–328 BCE) to the time of Wallerius (1709–1778 CE), the concept of oleum untuosm, equating fertile soil with the fatness of the land, dominated the ideas of naturalists. The word "humus" was extensively used in Virgil's (79–19 BCE) poetry about farming, food production, and the joys of country life. His poetry is extensively quoted relative to soil fertility, decomposition, gardening, nature, the environment, and organic agriculture, with the 39 BCE quote from the second Georgics

> "pinquis humus dulcique uliine laeta; Quique frequens hebis et fertilis übre campus"

being the most familiar. The word humus, together with terra and solum, was used for earth. It is the root word for humans, homo, and even posthumous, after the earth or death. Virgil referred to dark soil as fertile, and the ancients knew that dark-colored soil was more productive, absorbed more water, and was easier to till than its lighter colored counterparts in the landscape. They had also observed that exposure to flames often lightened the soil. Feller (1997) quotes Pliny the Elder (23–79 CE) as saying

> "the lupin penetrates the humus and wheat needs two feet of humus."

The period of alchemy and the phlogistic theory continued to use the original Latin definition of humus as soils or earth, as did Linnaeus (1707–1778), the great Swedish botanist. He classified soils as *Humus daedalea* (garden soil), *Humus rualis* (field soil), and *Humus latum* (muck soil). The concept that the application

of dung to the soil replaced some substances that had been removed by plants was established in the 16th century. Van Helmont's (1577–1644) experiments that concluded that water was the source of plant nutrition were repeated by Robert Boyle with the same conclusion. However, Woodward in 1699 showed that impure water, such as that from the river Thames, increased the growth of mint. He also reported that dung that returns parts of either vegetables or animals was the best way of restoring soil. Böerhavein, in a 1727 textbook of chemistry, wrote that plants absorb the juices of the earth. Tull in 1730 stated that small, earth-like particles serve as nutrients for plants.

Wallerius in 1753 (see Feller, 1997) used the Latin word humus for loam or mold, which at that time referred to the organic surface horizon relative to decomposing organic matter, and is thus credited with the modern use of humus for organic matter. This was made easier by the fact that the later Roman and Latin texts then utilized the word terra rather than humus for earth. Wallerius went along with the thinking of that time in assuming humus was the essential nutritive element and that other soil constituents acted in mixing or dissolving it and, thus, assisted uptake by plants. Lime was considered to help dissolve the fat (humus) of the land and the function of clay was to fix or retain this fatness. The Russian scientist Komov, in his 1782 book on agriculture, associated the hydrophysical properties of soil and its richness in nutrients with the presence of humus and stated that the "nutritive juice" of soil was produced by rotting.

De Saussure, known for his chemical studies, also spent considerable time on humus. In 1804, he described humus as being of various complexes (oils and salts), capable of absorbing oxygen and producing $CO_2$. He showed that it contained more C and less O and H than the plant residues that went into its formation. He also established that plants synthesize their organic matter from $CO_2$ and give off $O_2$. Thaer in 1808 differentiated between peat formed in limited $O_2$ and mild humus formed under adequate $O_2$. He ascribed to the humus theory of plant nutrition, which stated that humus was the direct source of plant nutrients. Thaer also has been called the father of sustainable agriculture (see Feller *et al.*, 2003b). One of his books stated,

> "Latterly the practice of sowing white clover with the last crop has become very general; only a few apathetic and indolent agriculturalists or men who are firmly wedded to old opinions and customs, neglect this practice."

It took the work of Sprengel in 1826, Liebeg in 1840, and Boussingault in 1841 (see Feller *et al.*, 2003b) to found the concept of mineral nutrition of plants. However, modern organic agriculture still credits soil organic matter with properties other than nutrient supply, water and nutrient retention, complexation, and aggregation. Humic constituents in small quantities continue to be investigated for their effect on plant respiration as does the use of specific plant- and microbial-derived molecules as information signals for plant and microbial interactions (Vaughn, 1985; Bais *et al.*, 2004).

Berzelius, first in 1806 and later in the 1830s, described the dark, black, and lighter yellow humic compounds and showed their interactions with metals. Field experiments carefully conducted in 1834 by Boussingault, considered the father of modern scientific agronomy, analyzed the C, H, O, N, and mineral inputs in manure relative to those in subsequent plant parts grown on manured soils. In 1826 and 1837, Sprengel found that the C content of humus is 58%, described the most important characteristics of humates (its salts), and studied their decomposition and solubility characteristics. The Russian scientist German, in 1837, still believed that humus was a direct source of plant nutrition, but found that cultivated soils contained less humus than virgin ones and attempted to obtain scientific confirmation of the value of rotations. This was a prelude to modern-day sustainable agriculture and the questions arising today regarding soil C and global change. He also was the first to question whether humic acids were chemically individual compounds. The large number of fractions he, and later others, identified as constituents of humus was not found to be reproducible and this led to a general questioning of the usefulness of soil organic matter fractionation. Danish scientist Müller (see Wilde, 1946) further defined the solubility and characteristics of humics in his book "Natural Forms of Humus" and developed the concepts of Mull and Mor in forest soils. Mull horizons had earthworms and fungi, whereas earthworms were absent from Mor soils.

Dokuchaiev, the founder of Western soil science, recognized the involvement of the five interacting factors of soil formation (parent material, vegetation, organisms, climate, and time) in the development of rich, high-organic-matter, chernozemic soils. Other scientists in this productive period include Kostychev, who in 1886 suggested that products synthesized by bacteria participated in the production of humic substances (see Kononova, 1961). Hebert in 1892 and Dehérain in 1902 developed the concept of humus formation as the interaction of lignin and proteinaceous substances. Büchner is credited for his pioneering work in enzymology by disrupting yeast cells to produce a cell-free system capable of alcoholic fermentation. This later led to the many investigations of enzyme reactions in soils.

During the period of 1908–1930, Shreiner, Shorrey, and their co-workers used large-scale extraction equipment to isolate 40 identifiable organics including hydrocarbons, sterols, fats, organic acids, aldehydes, carbohydrates, and organic P and N compounds. These studies gained a great deal of attention because of their precision, but may have detracted from the overall study of soil organic matter as a natural entity. They were a prelude to Waksman's detailed studies on the proximate analysis of organic matter in which he rejected the concepts of humic and fulvic acids. However, Tyurin in his 1937 book (see Kononova, 1961) on the organic matter of soils and Springer in 1934–1935 (see Kononova, 1961) regarded Waksman's denial of the existence of specific humic soil compounds as unfounded and incorrect, and claimed that proximate analysis, as suggested by Waksman, would not stand the test of time in that it characterized only a small fraction of humus. However, some mistrust of humic acid characterization, generated by Waksman's criticisms, continues today in Western soil science, although humic acid chemistry is well accepted in aquatic research in both marine and freshwater environments.

The translation of earlier Russian volumes entitled "Soil Organic Matter, Its Nature, Its Role in Soil Formation and Soil Fertility" (Kononova, 1961) described organic matter much as it is defined today and brought together literature on the role of physical, chemical, and biological factors of soil formation and its effect on cultivation. Stevenson's 1994 book entitled "Humus Chemistry" recognized the role of humic and fulvic acids and humic fractionation and delineated today's knowledge of organic C, N, P, and S transformations. Aiken *et al.* (1985) in "Humic Substances in Soil, Sediment and Water" recognize the similarity of humic substances in soils, sediments, and water. They describe methods, such as NMR and pyrolysis mass spectrometry, for studying this series of complex, and still difficult to study, soil organic matter constituents that form such an important component of present-day sustainable agriculture and global change investigations.

Nitrogen is important as a constituent of soil organic matter, as a nutrient in soil fertility, in water pollution, and in trace-gas, radiative forcing in global change. It thus continues to receive a great deal of attention. It took a great deal of research and many publications to delineate the processes of $N_2$ fixation and N immobilization, mineralization, plant uptake, and denitrification. The reviews edited by Bartholomew and Clark (1965), Stevenson (1982), and Mosier *et al.* (2004) delineate the use of instrumentation, tracers, and inhibitors in determining the processes and rates in soils. In 1943, Norman and Werkman labeled soybeans with $^{15}N$. Addition of the labeled residue to soil showed that 26% of the tagged N was recovered by a subsequent crop. Work with both $^{15}N$ and $^{13}C$ by Broadbent and Norman in 1946, and Broadbent and Bartholomew in 1948 (see Jansson, 1958; Paul and Van Veen, 1978), established the principles for the use of soil tracers. The equations of Kirkham and Bartholomew (1955) for mineralization–immobilization and the epic work of Jannson (1958) on soil N dynamics should be required reading for anyone today contemplating tracer studies.

The advent of tracers in the 1940s came at a time when the principles affecting plant decomposition had been reasonably established. Harmsen and van Schreven (1955) summarized the early work on the effects of environmental factors and the possibility of soil biota turnover in subsequent releases of N as follows:

> "The study of the general course of mineralization of organic N was practically completed before 1935. It is surprising that many of the modern publications still consider it worthwhile to consider parenthetical observations dealing with these entirely solved problems."

These authors then pointed out that the relationships between C and N and the effects of environmental factors had to be determined for each soil type, indicating that the underlying controls were not understood nor could the dynamics of resistant compounds be measured.

Libby developed the $^{14}C$ dating technique in 1952. It was used for peats, buried soil profiles, and soil pedogenesis by Simonart and Mayaudon in 1958, Simonson in 1959, and Tamm and Ostlund in 1960 (see Paul and Van Veen, 1978). In 1964,

Paul and co-workers carbon dated soil organic matter fractions to calculate their mean residence times. The further interpretation of carbon dating by Scharpenseel, and Stout and Rafter (see Goh, 1991), did a great deal to establish pools and fluxes for modeling purposes. Decomposition experiments with plant residues with laboratory-enhanced $^{14}C$ contents provided much information on the effects of soil type and climate management in studies by Sorensen in 1967, Jenkinson and Rayner in 1977, and Sauerbeck and Führ in 1968 (see Paul and Van Veen, 1978). Differences in naturally occurring $^{13}C$ resulting from C3 ↔ C4 plant vegetation switches and from enhanced $CO_2$ experiments are now being effectively utilized to answer global change and soil and ecological sustainability questions involving soil organic matter (Coleman and Fry, 1991; Boutton and Yamasaki, 1996).

The use of tracers allows one to also measure nontracer soil C and N. There is continual turnover of organic matter during decomposition, and tracer experiments often show more soil C and N being released than can be determined in the absence of the tracer. Some of today's authors are mistakenly calling this priming. Fontaine *et al.* (2004) credit Löhnis as defining priming in 1926 as an increased availability of nutrients due to higher microbial activity resulting from the addition of substrate. With the use of tracers, Broadbent and Bartholomew (1948) also defined priming as the increased mineralization of unlabeled soil organic matter constituents in the presence of available fertilizer N or labeled plant residues. Replacement by the tracer of nontracer C or N during normal soil dynamics must be taken into consideration before priming is said to occur. It is hoped that today's authors will read the original literature and not erroneously redefine what was established many years ago. Priming does occur. We must, however, use a mass balance approach together with the tracers to determine that it is a net release of the nutrients from soil organic matter and not a normal exchange of the tracer for nontracer isotopes during microbial growth and product formation.

There are excellent reviews on soil N, such as Bartholomew and Clark (1965), Stevenson (1994), and Mosier *et al.* (2004). These contain discussions of the significance of fixed ammonia as part of total soil N, especially with regard to depth, in clay soils. Today's literature seems to have forgotten this constituent. It is hoped that in the next 10 years, we will not read a spate of papers that claim to have newly discovered this not necessarily active, but important, N component.

Fred *et al.* (1932), Stewart (1975), and Graham (2000) have reviewed $N_2$ fixation. Prosser (1986) and Norton (2000) reviewed nitrification, whereas N losses, especially those leading to pollution and global warming, have been covered in Robertson (2000) and Groffman (2000). Publications such as "Biogeochemistry" (Schlesinger, 1997) and "Geomicrobiology" (Ehrlich, 1996) cover related areas of nutrient cycles and exchange in soils, freshwater sediments, and the vadose zone. The fact that the processes and process controls are similar in all environments is heartening for our level of knowledge. These controls lead to a rather similar composition for organic matter in most aerobic terrestrial soils. Modeling, such as that used by Jenkinson and Rayner (1977), is now an integral part of soil biochemistry used to test concepts and extrapolate information to different landscapes and for future predictions. Whether the ability to develop reasonably descriptive models based primarily on soil organic




FIGURE 1.2 A dog's eye view of decomposition and soil organic matter formation. Copyright 1962; reprinted by permission of United Feature Syndicate, Inc.

matter dynamics, but not soil population data, can be attributed to the great redundancy of microbial populations or to the fact that our models are not yet accurate enough to require population input data is yet to be determined. The 10-volume, edited series entitled "Soil Biochemistry" initiated by McLaren and Peterson (1967) has since been coedited by Paul and Ladd and by Stotsky and Bollag. It has brought together information on biologically related soil processes and components, nutrient cycles, and enzymes. It has also covered extraterrestrial life, soil enzymes, and pollutants as they affect soil organisms and the environment. The best way to summarize this section on soil biochemistry is to republish the cartoon from the comic strip Peanuts that was included in the first volume of the "Soil Biochemistry" series (Fig. 1.2).

## IN PERSPECTIVE

The soil microbiologist, ecologist, and biochemist must be aware that their organisms and processes are affected by soil type, vegetation, landscapes, and management. Forestry and rangelands are a very important component of our

studies. Wilde (1946), in his very readable book "Forest Soils and Forest Growth," quotes the following from the Kalevala, the National Epic of the Finns, dated to approximately 900 BCE, showing that early man recognized the interaction of soil type and vegetation.

> Seeds upon the land he scatters,
> Seeds in every swamp and meadow,
> Forest seeds upon the loose earth,
> On the firm soil he plants acorns,
> Spreads the spruce seeds on the mountains,
> And the pine seeds on the hill-tops,
> In the swamps he sows the birches,
> On the quaking marshes alders,
> And the basswood in the valleys,
> In the moist earth sows the willows,
> Mountain ash in virgin places,
> On the banks of streams the hawthorn,
> Junipers on knolls and highlands;
> Thus his work did Pellerwoinen. . . .

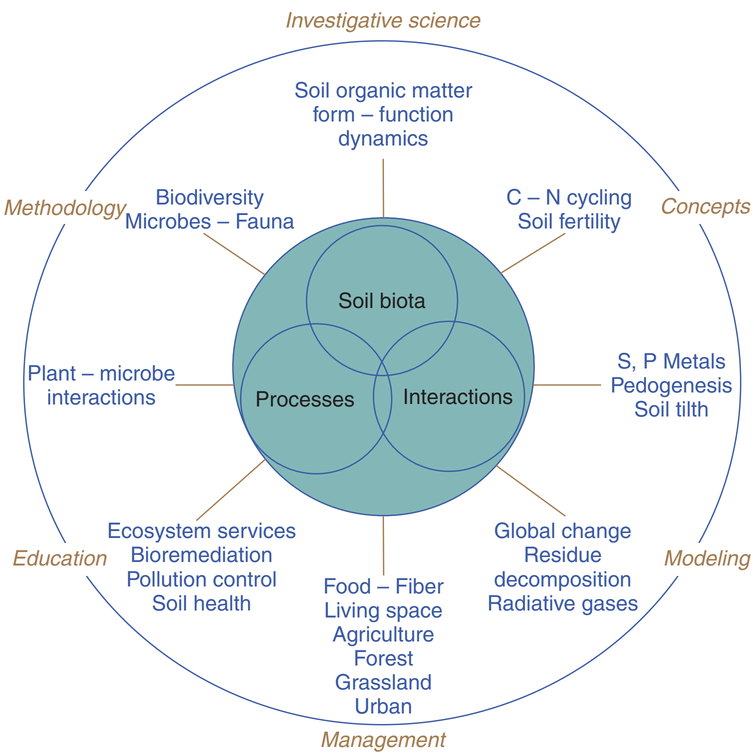



FIGURE 1.3 The interplay of soil biota, interactions, and processes in investigative science and management.

The great biodiversity of soil biota in both macro and micro forms, and the important questions that need to be answered, indicate to me that many of the new concepts in our field will come via the study of the physiology and ecology of soil organisms, as well the processes they mediate relative to soil nutrient transformations and global biogeochemical cycles. This text, therefore, has chapters on the physiology–biochemistry of organisms as well as on ecology in an attempt to enhance the understanding required to provide a foundation for the interdisciplinary approaches that will continue to provide exciting new concepts in our field. It is hoped that the individual chapters will provide new breakthroughs, concepts, methods, and ideas, as well as more individualized references. Figure 1.3 shows the interdependence of soil microbiology, ecology, and biochemistry, some of its fields of study, and some of its applications.

The last chapter in this volume will provide an oversight of the individual chapters and, it is hoped, provide insights into the future.

This edition is dedicated to that great soil microbiologist, F. E. Clark, whose keen insight and clear writing were such a joy to read in many early publications, as well as in the first two editions of "Soil Microbiology and Biochemistry."

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# 2

# The Soil Habitat

R. P. Voroney



At first sight nothing seems more obvious than that everything has a beginning and an end and that everything can be subdivided into smaller parts. Nevertheless, for entirely speculative reasons the philosophers of Antiquity, especially the Stoics, concluded this concept to be quite unnecessary. The prodigious development of physics has now reached the same conclusion as those philosophers, Empedocles and Democritus in particular, who lived around 500 BCE and for whom even ancient man had a lively admiration. (Svante Arrhenius, Nobel Lecture, 1903)

## Introduction

Soil is the naturally occurring, unconsolidated mineral and organic material at the earth's surface that provides an environment for living organisms. Recently, it has been referred to as the earth's "critical zone" and as deserving special status, because of its role in controlling the earth's environment and thus affecting the sustainability of life on the planet. This concept, that the earth's physicochemical properties are tightly coupled to the activity of the living organisms it supports, was proposed in the early 1970s by James Lovelock as the Gaia hypothesis. He theorized that the Earth behaved as a superorganism, with an intrinsic ability to control its own climate and chemistry and thus maintain an environment favorable for life. However, it is only microorganisms that have proven they can sustain the biosphere and can do so even without larger organisms.

The soil is where living organisms, or the biosphere, interact with rocks and minerals (geosphere), water (hydrosphere), atmosphere, and dead organic matter (detritosphere). Scientists study soil because of the fundamental need to understand the dynamics of geochemical–biochemical–biophysical interactions at the earth's surface, especially in light of recent and ongoing changes in global climate. What complicates this study is that while geochemical fluxes between the hydrosphere, atmosphere, and geosphere may take place over the time span of hundreds to millions of years, biologically induced fluxes between the geosphere, atmosphere, biosphere, and detritosphere take place over a much shorter time frame, hours and days to months.

The soil habitat is defined as the totality of living organisms inhabiting soil, including plants, animals, and microorganisms, and their abiotic environment. The exact nature of the habitat in which the community of organisms is living is determined by a complex interplay of geology, climate, and plant vegetation. This interaction of rock and parent material with temperature, rainfall, elevation, latitude, exposure to sun and wind, and many more factors, over broad geographical regions with similar environmental conditions and characteristic plant communities, has evolved into the current terrestrial biomes with their associated soils (Fig. 2.1).

Because soils provide such a tremendous range of habitats, they support an enormous biomass, with an estimated $2.6 \times 10^{29}$ prokaryotic cells alone, and harbor much of the earth's genetic diversity—a single gram of soil contains kilometers of fungal hyphae and more than $10^9$ bacterial cells, organisms belonging to tens of thousands of different species. Micro-zones of good aeration may be only millimeters from areas poorly aerated. Areas near the soil surface may be enriched with decaying organic matter and other nutrients, whereas the subsoil may be nutrient poor; the soil solution in some pores may be highly acidic, others more basic, depending on soil mineralogy and biological activity. Temperature and water contents of surface soil can vary widely from that of subsoils; and the microenvironment of the surfaces of soil particles, where nutrients are concentrated and water films vary in thickness, is very different from that of soil pores.

## SOIL GENESIS AND FORMATION OF THE SOIL HABITAT

By definition, soils are greater than 10 cm thick if formed from minerals and extend from the earth's surface into the underlying parent material from which they are formed. Soil may even be covered with water to a depth of up to 0.5 m as in coastal tidal marshes or inland water areas where bodies of periodically submerged soils merge into bodies of water in the natural landscape.

When plant residues are submerged in water for prolonged periods of the year and availability of $O_2$ is limited, biological decay is slowed and organic matter in various stages of decomposition accumulates. Deposits containing $>30\%$ organic matter and extending to depths of $\sim 0.5$ m or more form organic soils, and they include peatland, muck or bog, and fen soils.

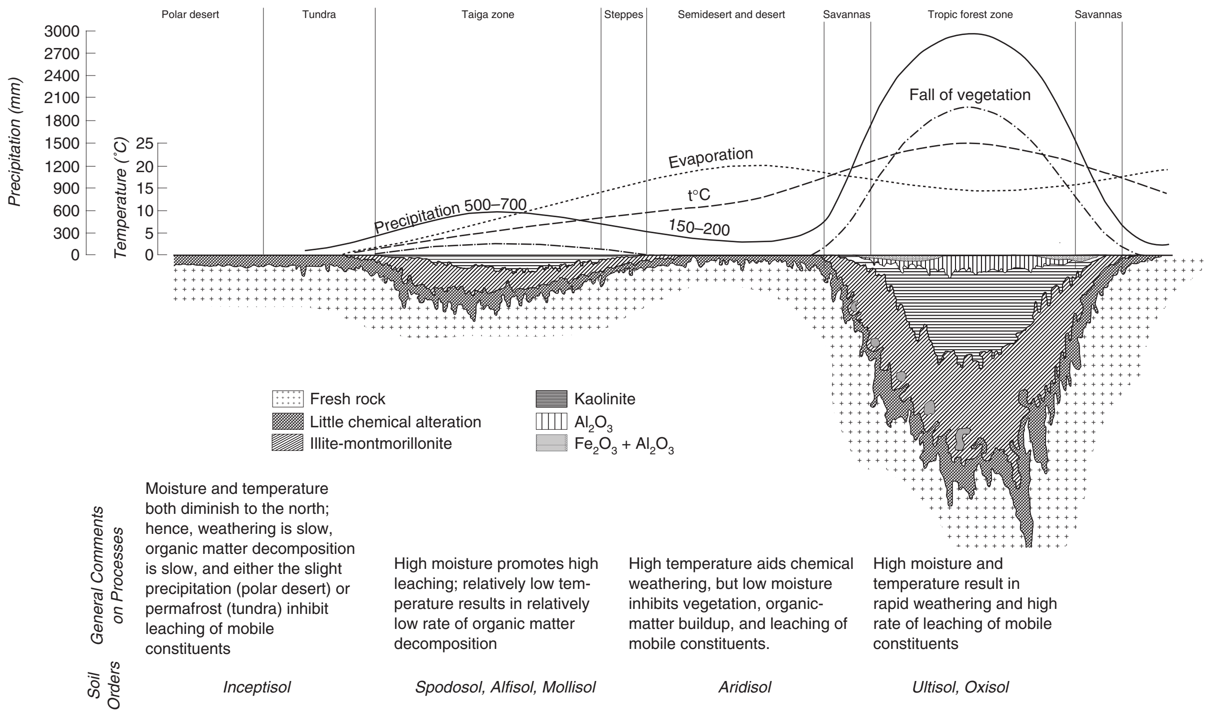



FIGURE 2.1 Environmental factors affecting the distribution of terrestrial biomes and formation of soils along a transect from the equator to the north polar region (with permission from Birkeland, 1999).

Mineral soils are formed by the physical and chemical weathering of the rocks and minerals brought to the earth's surface by geological processes. The parent material of mineral soils can be the residual material weathered from solid rock masses or the loose, unconsolidated materials that often have been transported from one location and deposited at another. The disintegration of rocks into smaller mineral particles is a physical process brought about by heating and cooling, freezing and thawing, and abrasion from wind, water, and ice masses. Chemical and biochemical weathering processes are enhanced by the presence of water, oxygen, and the organic compounds resulting from biological activity. These reactions convert primary minerals such as feldspars and micas to secondary minerals such as silicate clays, and they promote the release of constituent elements in soluble forms available to soil organisms and plants.

Physical and chemical weathering of rocks to fine particles with large surface areas and the accompanying release of plant nutrients initiate the soil-forming process (Fig. 2.2).

The initial colonizers of soil parent material are usually organisms capable of both photosynthesis and $N_2$ fixation. Early plant vegetation has intimate root–bacterial/fungal/actinomycetal associations with soil organisms to assist with supplying nutrients and water.

Soil organisms together with plants constitute one of the five interactive factors responsible for soil formation. By 1870, Russian soil scientists had developed the concept of soils as independent natural bodies each possessing unique properties resulting from parent material, climate, topography, and living matter, interacting

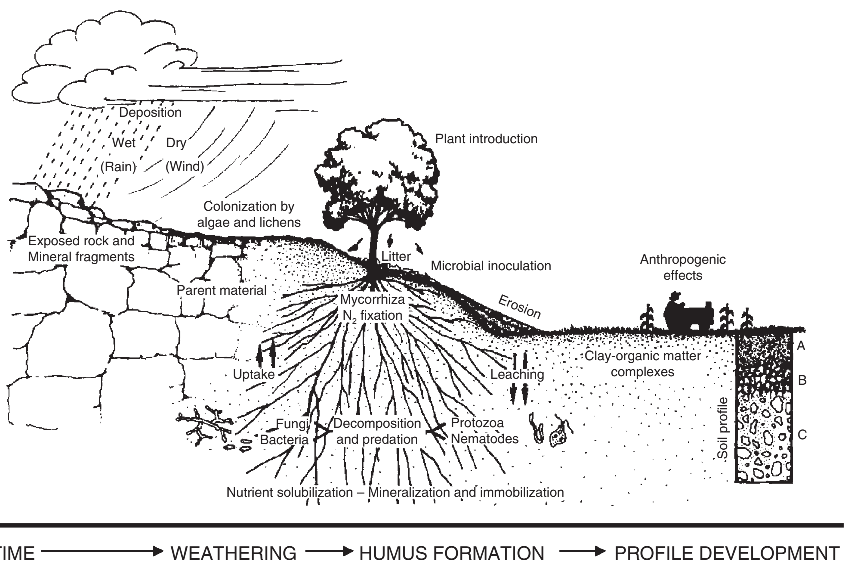



FIGURE 2.2 Interrelationships of organisms, organic matter, and parent materials in soil development.

over time. The approach to describing soil genesis as a biochemical product of organisms participating in their own genesis was quantified by Hans Jenny in his now classic equation of soil-forming factors:

$$\text{Soil} = f\,[\text{parent material, climate, living organisms, topography, time}].$$

## SOIL PROFILE

During formation soils develop horizontal layers, or horizons, that look different from one another (Fig. 2.3).

The horizons within a soil profile vary in thickness depending on the intensity of the soil-forming factors, though their boundaries are not always easy to distinguish. Uppermost layers of mineral soils are most altered during soil formation, whereas the deeper layers are most similar to the original parent material. Horizon differences in the solum, the parent material most altered during soil formation, involve: (i) organic matter from plant residues and roots in the surface mineral horizons decaying and forming humus, which gives these horizons a dark color—the organic-matter-enriched horizons nearest the soil surface are called A horizons; (ii) movement of soluble and colloidal inorganic and organic constituents from surface layers; and (iii) accumulation of varying amounts of inorganic and organic precipitates. These underlying, enriched layers in mineral soils are referred to as B horizons. The C horizons are the least weathered part of the mineral soil profile. Organic soils are commonly saturated with water and consist mainly of mosses, sedges, or other hydrophytic vegetation; the upper material is referred to as the O layer. In upland areas where drainage is better and forest vegetation supported, folic-derived organic materials accumulate to form the L–F–H layer. In both types of organic soils, it is the residual organic matter in the surface layer that most resembles the vegetation from which it is derived.

The vadose zone is the underlying, unsaturated, parent material extending (from the soil surface) downward to where it reaches the water table and the soil becomes saturated. Below the solum, this zone contains relatively unweathered parent material, low in organic matter and nutrients and often deficient in $O_2$. The thickness of the vadose zone can fluctuate considerably during the season, depending on soil texture, soil water content, and height of the soil water table. When the water table is near the surface, for example as in wetlands, it may be narrow or nonexistent. But in arid or semiarid areas where soils are well drained, the vadose zone can extend for several meters and even be hundreds of meters deep.

# PHYSICAL ASPECTS OF SOIL

Dimensions of physical features commonly encountered when considering the soil habitat range from meters (pedon, soil landscape, and watershed), down through a few millimeters (the fine-earth fraction), to a few micrometers (microorganisms and clay minerals) and nanometers (humic molecules) (Table 2.1).

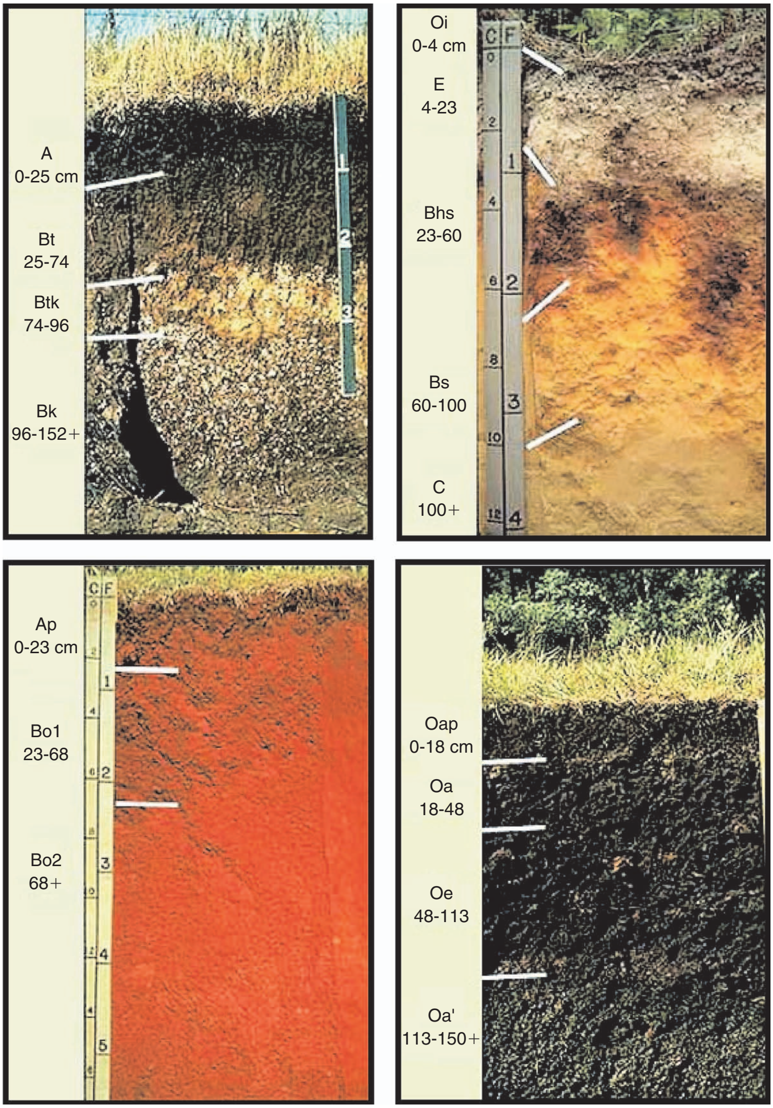



FIGURE 2.3 Natural (undisturbed) mineral and organic soils showing the soil profile: mollisol (top left), spodosol (top right), oxisol (bottom left), and histosol (bottom right). (Soil profiles reproduced with permission: spodosol and oxisol from University of Nebraska Press, mollisol from American Society of Agronomy; histosol from University of Idaho.)

## SOIL TEXTURE

The larger mineral particles include stones, gravels, sands, and coarse silts that are generally derived from ground-up rock and mineral fragments. While particles >2 mm in diameter may affect the physical attributes of a soil, they are excluded

TABLE 2.1 Spatial Dimensions of Features Commonly Encountered in Describing the Soil Habitat

| Scale (m) | Particles | Aggregations | Pore (functions) | Biota | Scale (m) |
|---|---|---|---|---|---|
| $10^{-10}$ | Atoms | | | | $10^{-10}$ |
| | | Amorphous minerals | MICROPORES | | |
| $10^{-9}$ | Molecules | | | Organic molecules | $10^{-9}$ |
| | | | (Adsorbed and intercrystalline water) | Poly-saccharides | |
| $10^{-8}$ | Macromolecules | | | Humic substances | $10^{-8}$ |
| | Colloids | Clay micro-structure | | Viruses | |
| $10^{-7}$ | | | $\psi > -1500$ kPa | - - - - - - - - - - | $10^{-7}$ |
| | | | | Bacteria | |
| | Clay particles | Quasicrystals | MESOPORES | | |
| $10^{-6}$ | | Domains | | Fungal hyphae | $10^{-6}$ |
| | Silt | Assemblages | (Plant available water) | | |
| $10^{-5}$ | | | | Root hairs | $10^{-5}$ |
| | | Micro-aggregates | $\psi < -10$ kPa | | |
| $10^{-4}$ | Sand | | MACROPORES AND CRACKS | Roots-Mesofauna | $10^{-4}$ |
| $10^{-3}$ | | | (Aeration) | | $10^{-3}$ |
| | | Macro-aggregates | (Fast drainage) | | |
| $10^{-2}$ | Gravel | | | Worms | $10^{-2}$ |
| $10^{-1}$ | | | | Moles | $10^{-1}$ |
| | Rocks | Clods | | | |
| $10^{-0}$ | | | | | $10^{-0}$ |

from the definition of soil. The fine-earth fraction of soil particles ranges in size over four orders of magnitude: from 2.0 mm to smaller than 0.002 mm in diameter. Sand-sized particles are individually large enough (2.0 to 0.05 mm) to be seen by the naked eye and feel gritty when rubbed between the fingers in a moist state. Somewhat

smaller, silt-sized particles (0.05 to 0.002 mm) are microscopic and feel smooth and slippery even when wet. Clay-sized particles are the smallest of the mineral particles ($<$0.002 mm), seen only with the aid of an electron microscope, and when wet form a sticky mass. The proportion of these different size classes in soil is referred to as soil texture, and terms such as sandy loam, silty clay, and clay loam are textural classes used to identify the soil's texture. When investigating a field site, considerable insights into the behavior and properties of the soil can be inferred from its texture, so it is often one of the first properties to be measured.

The surface of mineral soils contains an accumulation of living biomass, dead and decomposing organic material, and humus. This soil organic matter (SOM) typically accounts for 1–10% of the total soil mass, but because it is intimately associated with the mineral fraction it is difficult to isolate from the soil. The larger, recognizable remains of plant, animal, and soil organisms that can be separated from soils by hand picking and sieving techniques are referred to as particulate organic matter. These tissues undergo continuous decay, and over periods of years to decades, brown to black-colored, colloidal humus is synthesized and accumulates. Soil humic substances can account for 50–60% of the total SOM, and together with the nonhumic material provide a nutrient reservoir to sustain the soil microbial biomass.

## SOIL STRUCTURE

Typically, the individual mineral particles in surface soils are coated and glued together with colloidal organic matter and encrusted with inorganic cements forming spatial clusters within the matrix known as aggregates or peds. In 1982, two Australian soil scientists, Drs. Judy Tisdall and Malcolm Oades, presented a conceptual model of the aggregated, hierarchical nature of the soil system and described the linkages between the architecture of the soil habitat and the role of microbial activity in its genesis. If soil was but a single ped, scientists could describe its physical and chemical properties in sufficient detail to understand how the nature and activity of soil organisms are controlled. But soils are composed of highly variable peds, derived from a wide range of parent materials that exist within innumerable landscapes, and exposed to diverse climates. And they have formed in concert with the development of the complex communities of living organisms that make up the biosphere.

Overall, the shape, size, and arrangement of the aggregates within the soil profile describe soil structure. Three assemblages of aggregates are recognized with diameter classes of 0.002–0.020, 0.020–0.250, and $>$0.250 mm and are referred to as microaggregates, mesoaggregates, and macroaggregates, respectively.

Microaggregates are formed by flocculation of fine silt and clay particles, amorphous minerals (composed of oxides and hydroxides of aluminum, silicon, iron, and manganese and silicates of aluminum and iron), and nonhumic and humic substances, largely dominated by electrostatic and van der Waal forces. Polyvalent cations such as $Al^{3+}$, $Fe^{3+}$, $Ca^{2+}$, and $Mg^{2+}$ adsorbing onto their surfaces and

reacting with exposed functional groups promote these flocculation reactions. Sticky polysaccharides and proteins derived from plant and animal tissues, microbial cells, and exudates from roots, hyphae, and bacteria further enhance these stabilization reactions. In particular, soil microorganisms produce extensive exopolysaccharides, which they use to adhere to individual soil particles.

The core of mesoaggregates is usually the residual debris left from the decay of plant and microbial tissues. Bits of decaying particulate organic matter and their colonizing microbial biofilms become encrusted with fine mineral particles and they act as nuclei for the formation of aggregates and contribute to their resilience. Microaggregates can form throughout the surface soil matrix wherever intense microbial activity associated with organic matter decomposition occurs. The larger macroaggregates are formed only where a network of living and decaying plant roots, fungal hyphae, and microbial filaments physically enmesh clusters of micro- and mesoaggregates for a period sufficient for them to be chemically linked.

In the rhizosphere, hyphae of arbuscular mycorrhizal fungi contribute to the aggregation effect as they grow into small pores and bind soil particles together. Although macroaggregates comprise microaggregates, not all soil microaggregates exist as macroaggregates. Macroaggregates can contain soil primary particles that may eventually go on to form microaggregates. This hierarchical organization of soil structure and aggregation, i.e., large aggregates being composed of smaller aggregates, which in turn are composed of even smaller aggregates, is characteristic of most soils (Fig. 2.4).

Micro- and mesoaggregates tend to be especially resistant to mechanical breakdown, for example, from the impact of rainfall or from slaking—rapid rewetting of dry soil—or from freezing and thawing. The restricted size of the pores within these aggregates ($<0.01$ mm diameter), also referred to as intra-aggregate pore space, can limit the interactions of soil organisms. The pore space surrounding microaggregates and contained within macroaggregates is collectively referred to as the soil interaggregate pore space. It contains plant roots and is usually rich in fresh inputs of particulate macroorganic matter ($>0.05$ mm diameter). Macroaggregates usually remain intact as long as the soil is not disturbed, for example, by earthworm and other faunal activity or by disturbance such as heavy rains. Macroaggregation is important for controlling microbial activity and soil organic matter turnover in surface soils because it gives fine-textured, clayey and loamy-textured soils pore space characteristics similar to those of sandy soils. The physical properties affected by macroaggregation include pore size distribution, pore continuity and tortuosity (irregular, twisted pores), aeration, drainage, and tilth.

## SOIL HABITAT SCALE AND OBSERVATION

### SCALE OF SOIL HABITAT

The soil habitat is characterized by heterogeneities across all measured scales, from nanometers to kilometers, which differ in chemical, physical, and biological

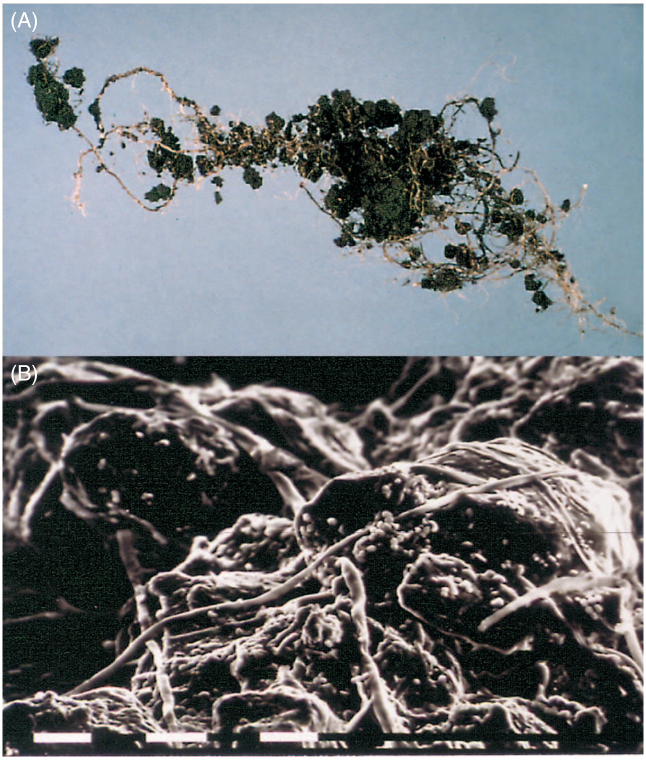



FIGURE 2.4 Hierarchical model of soil aggregation and binding agents functioning for aggregation. Larger aggregates are often composed of an agglomeration of smaller aggregates. Different factors are important for aggregation at each of the levels in the hierarchy of soil aggregates. (A) A *macroaggregate* composed of many microaggregates bound together mainly by a sticky network formed from fungal hyphae, bacterial cells, and fine roots. (B) A *microaggregate* consisting mainly of fine sand grains and smaller clumps of silt grains, clay, and organic debris bound together by root hairs, fungal hyphae, and microbial biofilms. Submicroaggregates consist of fine silt particles encrusted with organic debris and bits of plant and microbial debris (called *particulate organic matter*) encrusted with even smaller packets of clay, humus, and Fe or Al oxides. White bar indicates 10 μm. (Photos courtesy of D. A. Angers, C. Chenu, and S. Recous.)

characteristics in both space and time (Table 2.1). At various levels within this continuum of scales, different soil properties used to characterize the soil habitat can assume greater or lesser importance depending on the function or attribute that is under consideration. For broad general issues, such as climate change at a global scale, the general distribution of broad hydrologic features may be appropriate. Evaluations of more specific processes impacting on individual species' functioning may be possible only at the scale of habitat niches that occur at microsites in soil.

The habitat includes the physical location where a particular organism resides as well as the characteristics of the habitat that influence the growth, activities,

interactions, and survival of other organisms found in it. Together these characteristics define the range of habitats available for organisms and their enzymes to occupy. They vary vertically, through the soil vadose zone, and horizontally across the landscape.

For higher organisms, such as animals that range over wide territories, the habitat may be on the scale of a landscape or watershed and even beyond. At the other extreme, microorganisms, the habitat occurs on a microscale and therefore has been referred to as microhabitat. The spatial characteristics of the general habitat, as well as the fine features of the microhabitats, must be considered in describing the activity of soil microorganisms. Microregions within soil aggregates control the nature and availability of nutrient resources, directly impacting population dynamics. Metabolically similar groups of microorganisms, referred to as guilds, occupy a unique ecological niche that encompasses their life strategy. Sets of guilds, which carry out interdependent physiological processes, form microbial communities. While ecological theory has proposed that no two species ever occupy exactly the same ecological niche for long, in soils it is the rule that different species share aspects of their niche with others. Soil habitat spatial heterogeneity is an important contributor to the coexistence of species in soil microbial communities, enhancing overall soil biodiversity by promoting the persistence of individual populations.

Studies have confirmed that soil organisms are usually not randomly distributed but exhibit predictable spatial patterns over wide spatial scales (Fig. 2.5).

Spatial patterns of soil biota also affect the spatial patterns of microbial activity and processes they carry out. Accumulations of inorganic N may not be observed where sites of plant residue decay are adjacent and closely linked to those where ammonification of SOM is occurring. Inorganic N can accumulate and nitrification can occur where these microbial processes are physically separated in soil space. Recent studies of “trigger molecules” have identified substrates that appear to promote metabolic linkages over spatially diverse soil microbial communities (De Nobili *et al.*, 2001).

Although the main factors influencing the gross behavior of soil organisms are known, their relative importance and influence on spatial distribution have not been studied in detail. For soil microorganisms, there are few methods currently available that enable the study of their detailed activity *in situ* at the level of the soil microhabitat. In fact, when collecting samples in the field from a soil profile, it is common practice for soil scientists to homogenize the samples before analysis, after removal of the plant debris and macrofauna, by passing the soil through a 2-mm sieve.

## PORE SPACE

Between the solid (mineral and organic) components of soil is space forming pores that vary in size. On a volume basis, mineral soils are about 35–55% pore space, whereas organic soils are 80–90% pore space. But total soil pore space can

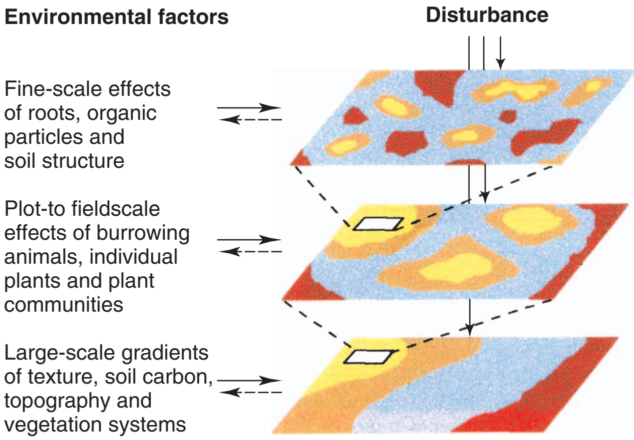



FIGURE 2.5 Determinants of spatial heterogeneity of soil organisms. Spatial heterogeneity in soil organism distributions occurs on nested scales and is shaped by a spatial hierarchy of environmental factors, intrinsic population processes, and disturbance. Disturbance operates at all spatial scales and can be a key driver of spatial heterogeneity, for example, through biomass reduction of dominant organisms or alteration of the physical structure of the soil substrate. Feedback between spatial patterns of soil biotic activity and heterogeneity of environmental factors adds further complexity (dotted arrows) (from Ettema and Wardle, 2002, with permission from Elsevier).

vary widely for a variety of reasons, including soil mineralogy, bulk density, organic matter content, and disturbance. Pore space can range from as low as 25% for compacted subsoils in the lower vadose zone to more than 60% in well-aggregated clay-textured surface soils. Sandy-textured soils, though having a higher mean pore size, tend to have less total pore space than do clay soils.

Soil pore space is defined as the percentage of the total soil volume occupied by soil pores:

$$\% \text{ pore space} = (\text{pore volume/soil volume}) \times 100. \tag{2.1}$$

Direct measurement of the soil pore volume is somewhat tedious to carry out; it is usually estimated from data on soil bulk density and soil particle density using the following formulas:

$$\text{soil bulk density—}D_b\ (\text{Mg/m}^3) = \text{soil mass (Mg)/soil bulk volume (m}^3\text{)}, \tag{2.2}$$

$$\text{soil particle density—}D_p\ (\text{Mg/m}^3) = \text{soil mass (Mg)/soil particle volume (m}^3\text{)} \tag{2.3}$$

(usually assumed to be 2.65 $Mg/m^3$ for silicate minerals, but can be as high as 3.25 $Mg/m^3$ for iron-rich tropical soils and as low as 1.3 $Mg/m^3$ for volcanic soils and organic soils),

$$\% \text{ pore space} = 100 - [(D_b/D_p) \times 100]. \tag{2.4}$$

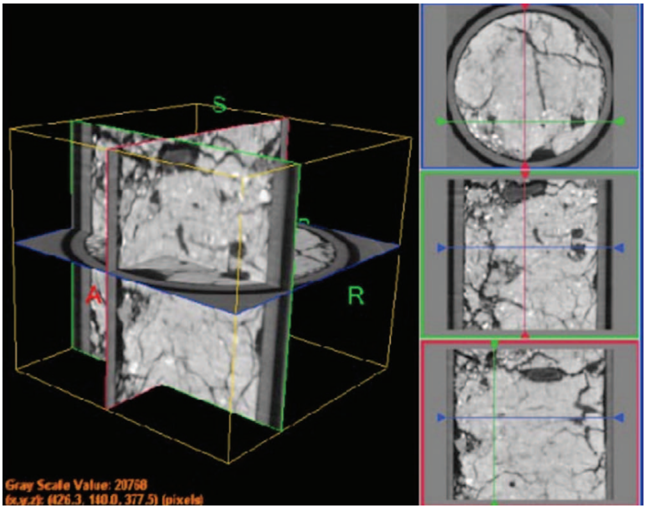



FIGURE 2.6 A 3-D CT X-ray scan of a soil core 6.4 cm in diameter × 10 cm in length, with a resolution of 10 μm, showing pore space distribution. (Courtesy of R. Heck, University of Guelph.)

Though total pore space is important, fundamentally it is the size, shape, and interconnection of soil pores that are the key in determining the habitability of the soil, for they directly control soil aeration and water relationships; this is largely a function of soil texture and structure (Fig. 2.6).

Total pore space is usually divided into two size classes, macropores and micropores, based on their ability to retain water after water drainage under the influence of gravity (Table 2.2). Macropores are those pores larger than ~10 μm in diameter that allow rapid diffusion of air and rapid water infiltration and drainage. They can occur as the spaces between individual sand and coarse silt grains in coarser-textured soils and in the interaggregate pore space of well-structured loam- and clay-textured soils. Macropores can also be created by roots, earthworms, and other soil organisms, forming an important type of pore termed "biopore." Biopores are typically lined with cutans rich in organic matter and clay and are ideal habitats for soil microorganisms. They provide continuous channels extending throughout the soil, often for lengths of a meter or more.

Soil pores less than 10 μm in diameter are referred to as micropores. Though important for retention of water available for plants and providing an aqueous habitat for microorganisms, the restricted size of micropores can limit interactions of soil organisms and their access to potential substrates.

While the larger micropores together with the smaller macropores can accommodate plant root hairs and microorganisms, pores smaller than ~5 μm in diameter are not habitable by most microorganisms and may even be too small to permit entrance of their exoenzymes.

TABLE 2.2 Pore-Size Characteristics across a Textural Range and Their Relation to Soil Water Potential (% of total soil porosity)

| | | | Textural classes | | |
|---|---|---|---|---|---|
| General pore categories | Soil water potential (kPa) | Equivalent diameter (μm) | Sandy loam | Loam | Clay loam |
| Macropores | 0 | Pores filled | | | |
| | –1 | ⩽300 | 5 | 3 | 2 |
| Micropores | –10 | ⩽30 | 15 | 8 | 2 |
| | –100 | ⩽3 | 3 | 16 | 10 |
| | –1,000 | ⩽0.3 | 2 | 9 | 17 |
| | –10,000 | ⩽0.03 | 1 | 5 | 15 |
| Total pore space (%): | | | 28 | 45 | 58 |

Molecular diffusion dominates the transport of gases in the soil. Diffusion through the air-filled pores maintains the gaseous exchange between the atmosphere and the soil, and diffusion through water films of varying thickness maintains the exchange of gases with soil organisms. Diffusion through both pathways can be described by Fick's law,

$$J = -D\, dc/dx,$$

where $J$ is the rate of gas diffusion (g cm$^{-2}$ sec$^{-1}$), $D$ is the diffusion coefficient (cm$^2$ sec$^{-1}$), $c$ is the gas concentration (g cm$^{-3}$), $x$ is the distance (cm), and $dc/dx$ is the concentration gradient.

The diffusion coefficient in soil is much smaller than that in air because of the limited fraction of total pore volume occupied by continuous air-filled pores and pore tortuosity, soil particles, and water, reducing the cross-sectional area and increasing the mean path length for diffusion. It is referred to as the effective diffusion coefficient, $D_e$, and is a function of the air-filled porosity. In addition to the diffusive path in the air phase of the soil, diffusion of gases in water is ~1/10,000 of that in air (Table 2.4). Thus, gaseous diffusion through a 10-μm water film offers the same resistance as diffusion through a10-cm air-filled pore.

The work of Kubiena in the 1930s contributed significantly to our understanding of the nature of soil solid and pore space at the microscopic scale. Much of this early work was based on the examination of thin sections (25 μm thick) of intact blocks of soil. Adaptation of advancements in the acquisition and computer-assisted analysis of digital imagery during the past quarter century have led to the quantitative spatial analysis of soil components. Thin sections represent only a single slice of soil so it is practically impossible to extrapolate observations accurately into three dimensions. Recent developments in microcomputerized X-ray tomography (CT scanning) allow study of the properties of the soil's intact three-dimensional

structure. These systems have resolution capabilities down to 10 μm, which allow differentiation of solids, and are able to quantify the distribution of organic and mineral materials. The technology is also able to readily distinguish air-filled and water-filled pore space. Distinguishing microbes from soil particles with this technology, however, is still not possible. A CT image of a soil core in three orthogonal planes is shown in Fig. 2.6. Highly attenuating features like iron oxide nodules appear bright in the imagery, while features with low attenuation capability such as pore space appear dark.

## SOIL SOLUTION CHEMISTRY

An understanding of the chemistry of the soil solution providing an environment for soil organisms needs to take account of the nature and quantity of its major components: water, dissolved organic matter and inorganic constituents, and $O_2$ and $CO_2$. The biogeochemistry of the soil solution is mainly determined by acid–base and redox reactions. Consequently the thermodynamic activities of protons and electrons in soil solution define the chemical environment that controls microbial activity. Both can be considered as flowing from regions of high concentration to regions of low concentration, and soil microbial activity has a profound effect on regulating this flow.

The most reduced material in the biosphere is the organic matter contained in living biomass. Organic matter in soils ranges from total dominance, as in peatlands, to the minor amounts found in young soils or at depth in the vadose zone. Soil organisms generate electrons during the metabolic oxidation of organic matter, and these electrons must be transferred to an electron acceptor, the largest of which is atmospheric $O_2$ in freely drained, aerobic soils. The $O_2$ trapped in the soil or present in the water can be consumed within hours by soil microbes and is replenished by $O_2$ diffusion. If $O_2$ diffusion into the soil is impeded, for reasons of waterlogging, restricted pore sizes due to clay texture or to soil compaction, the resultant soil becomes practically devoid of $O_2$. When microbial activity uses up all of the available dissolved $O_2$, the soil solution as a whole changes from aerobic (oxic) to anaerobic (anoxic). Microbial activity will then be controlled by the movement of electrons to alternative electron acceptors.

Development of anaerobic conditions results in a shift in the activity of the soil microbial populations, with the activity of aerobic and facultative organisms, which dominate well-drained soils, decreasing and the activity of obligate anaerobic and fermentative organisms increasing. This switch in electron acceptors promotes the reduction of several important elements in soil, including nitrogen, manganese, iron, and sulfur, in a process known as anaerobic respiration and carbon dioxide by methanogenisis.

Redox potential ($E_H$) measurements provide an indication of the soil aeration status. They are a measure of electron availability occurring as a result of electron transfer between oxidized (chemical species that have lost electrons) and reduced (chemical species that have gained electrons) chemical species. The measurements

TABLE 2.3 The Most Important Redox Pairs and the Approximate $E_H$ Values at the Occurrence of Transitions at the Reference Soil pH of 7.0

| | Oxidized form | Reduced form | Approximate $E_H$ at transformations (mV) |
|---|---|---|---|
| Oxygen | $O_2$ | $H_2O$ | +600 to +400 |
| Nitrogen | $NO_3^-$ | $N_2O$, $N_2$, $NH_4^+$ | 250 |
| Manganese | $Mn^{4+}$ | $Mn^{2+}$ | 225 |
| Iron | $Fe^{3+}$ | $Fe^{2+}$ | +100 to –100 |
| Sulfur | $SO_4^{2-}$ | $S^{2-}$ | –100 to –200 |
| Carbon | $CO_2$ | $CH_4$ | Less than –200 |

are often used to predict the most probable products of biological reactions. For example, $N_2O$ can be produced from nitrification under aerobic conditions and denitrification under moderately reducing conditions, where the reduction intensity is not strong enough to reduce nitrate completely to $N_2$ gas.

The magnitude of $E_H$ depends on $E^0$ and also on the relative activities of the oxidant and the reductant. These quantities are related by the Nernst equation:

$$E_H\,(V) = E^0 - (0.0591/n)\log(\text{reduct})/(\text{oxid}) + (0.0591m/n)\,\text{pH}.$$

The $E_H$ is the electrode potential of the standard hydrogen electrode, $E^0$ is the standard half-cell potential, $n$ is the number of electrons transferred, $m$ is the number of protons exchanged, reduct is the activity of the reduced species, and oxid is the activity of the oxidized species.

The major redox reactions occurring in soils and the electrode potentials for these transformations are shown in Table 2.3. Typically, dissolved $O_2$ and $NO_3^-$ serve as electron acceptors at $E_H \sim 350$ to 400 mV and above, until their concentrations in the soil solution drop to about 0 at 350 mV. Manganese and Fe serve as electron acceptors starting around 350 mV for Mn and 250 mV for Fe to ~100 mV. When $Fe^{3+}$ in Fe oxide is reduced, the oxide dissolves and $Fe^{2+}$ goes into solution. Sulfate reduction occurs from $E_H$ as high as 350 to ~100 mV. Methane production begins when $E_H$ is close to ~100 mV. Manganese, Fe, and $SO_4^{2-}$ reduction processes occur over a much wider range compared with $O_2$ and $NO_3^-$ reduction and methane production.

Although the activity of electrons can be described by p$E$, $E_H$ has the advantage of being a standard measurement for investigations of soil redox potential both in the laboratory and in the field. Soil $E_H$ can be obtained relatively easily from measurements of the pore water using a platinum (Pt) electrode. It is important to ensure that the electrodes are functioning properly and maintain performance, i.e., cleaned and calibrated before being installed in the soil and checked at regular intervals to ensure that they not become contaminated by surface reactions and lose their efficiency or accuracy.

**TABLE 2.4** Temperature Effects on Gaseous Diffusion in (A) Air and (B) Water and (C) Solubility in Water

| Temperature (°C) | $N_2$ | $O_2$ | $CO_2$ | $N_2O$ |
|---|---|---|---|---|
| (A) Gaseous diffusion coefficients in air ($cm^2/sec$) | | | | |
| 0 | 0.148 | 0.178 | 0.139 | 0.179 |
| 10 | 0.157 | 0.189 | 0.150 | 0.190 |
| 20 | 0.170 | 0.205 | 0.161 | 0.206 |
| 30 | 0.180 | 0.217 | 0.172 | 0.218 |
| (B) Gaseous diffusion coefficients in water ($\times 10^{-4}$ $cm^2/sec$) | | | | |
| 0 | 0.091 | 0.110 | 0.0876 | 0.111 |
| 10 | 0.130 | 0.157 | 0.125 | 0.158 |
| 20 | 0.175 | 0.210 | 0.167 | 0.211 |
| 30 | 0.228 | 0.275 | 0.219 | 0.276 |
| (C) Solubility coefficients (volume of dissolved gas relative to volume of water, $cm^3/cm^3$) | | | | |
| 0 | 0.0235 | 0.0489 | 1.713 | 1.30 |
| 10 | 0.0186 | 0.0380 | 1.194 | 1.01 |
| 20 | 0.0154 | 0.0310 | 0.878 | 0.71 |
| 30 | 0.0134 | 0.0261 | 0.665 | 0.42 |

Soil $E_H$ can be a difficult parameter to interpret. The Pt electrode measurement may not reflect changes in some species involved in redox reactions, such as the partial pressure of $O_2$. The presence of Mn, Fe oxides, and nitrates does not have the expected quantitative effect on the Pt electrode measurement. Methane, bicarbonate, $N_2$ gas, nitrate, and sulfate are not electroactive, i.e., they do not readily take up or give off electrons at the surface of the Pt electrode. Since it is a measurement of potential, the Pt electrode also responds to changes in pH and other potentials. Often two or more redox reactions occur simultaneously, thus measured $E_H$ usually reflects a mixed potential.

Nevertheless, platinum-electrode $E_H$ measurements are still useful and can be interpreted as a semiquantitative assessment of a soil's redox status. In studies of paddy soils, for example, $E_H$ measurements can be used to monitor progressive development of reducing conditions and can distinguish oxic and anoxic conditions.

Rice fields provide a unique aerobic and anaerobic environment to study the relationship between soil $E_H$ and greenhouse gas emissions because of controlled irrigation and drainage practices (Fig. 2.7).

During the flood season the paddy soils are a major source of $CH_4$, but an important source of $N_2O$ when they are drained. Strategies designed to mitigate $CH_4$ emissions from submerged rice fields can adversely affect greenhouse warming potential by stimulating higher $N_2O$ emissions. The different $E_H$ conditions required for $N_2O$ and $CH_4$ formation and the trade-off pattern of their emissions as found in rice fields make it a challenge to abate the production of one gas without enhancing the production of the other. Figure 2.7 shows the redox window offering the minimum global warming potential contribution from rice soils.

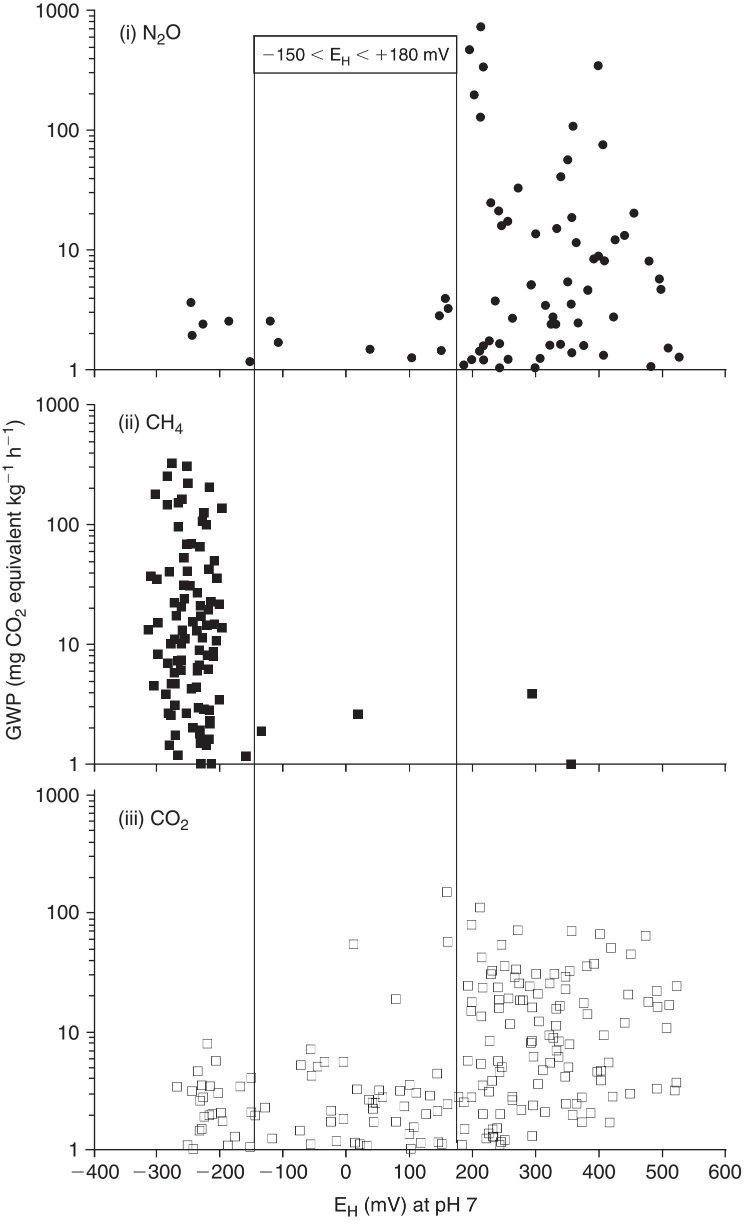



FIGURE 2.7 Global warming potential (GWP) contribution of $N_2O$, $CH_4$, and $CO_2$ as a function of soil $E_H$. All eight soils showed the same pattern of (i) $N_2O$, (ii) $CH_4$, and (iii) $CO_2$ dynamics with soil $E_H$ change from high to low. A logarithmic scale is used to cover a wide range of values. Global warming potential contributions below 1 mg $CO_2$ equivalent $kg^{-1}$ $h^{-1}$ were considered insignificant and not illustrated for clarity (from Kewei and Patrick, 2004.)

## SOIL pH

Acidic protons supplied to the soil from atmospheric and organic sources react with bases represented by aluminosilicates, carbonates, and other mineralogical and humic constituents. In a humid climate with excess precipitation, and given sufficient time, basic cations ($Na^+$, $K^+$, $Ca^{2+}$, $Mg^{2+}$) will be exchanged from mineral and organic constituents by $H^+$ and leached from the surface soil. The presence of clay minerals such as smectites, which are saturated with basic cations, retards the progress of acidification, and calcite buffers the soil between pH 7 and 8 until it is completely dissolved. Continued hydrolysis results in the formation of a residuum made up of such minerals as kaolinite, gibbsite, and goethite, together with resistant minerals, of which quartz is common, that are buffered between pH 3.5 and 5. Semiarid and arid conditions lead to an opposite trend, a soil solution buffered at an alkaline pH and in the presence of sodium.

Soil pH influences a number of factors affecting microbial activity, like solubility and ionization of inorganic and organic soil solution constituents, and these will in turn affect soil enzyme activity. There are a large number of both organic and inorganic acids found in soils; the majority of these acids are relatively weak. Traditionally soil pH is measured in a soil paste prepared by the addition of a dilute $CaCl_2$ solution with an appropriate electrode. While achieving a pH measurement of the soil is relatively easy, interpretation of its affect on microbial processes is difficult. This is because concentrations of cations sorbed to the surfaces of the negatively charged soil colloids are 10–100 times higher than those of the soil solution. The pH at the colloid surface will be much more acidic than that of the measured pH of the bulk soil solution. For soil exoenzymes sorbed to colloid surfaces, their apparent pH optimum would be 1–2 pH units higher than if measured free in solution. An example of this is soil urease activity, which has an apparent pH optimum of 8.5–9.0 that is about 2 pH units greater than optimal urease activity measured in solution.

## SOIL TEMPERATURE

Many physical, chemical, and biological processes that occur in soil are influenced by temperature. Increasing temperature enhances mineralization of SOM or decomposition of plant residues by increasing rates of physiological reactions and by accelerating diffusion of soluble substrates in soil. An increase in temperature can also induce a shift in the composition of the microbial community. Whereas rates of molecular diffusion always increase with increasing temperature, solubility of gases in soil solution do not, and can even decrease, thereby slowing microbial activity (Table 2.4).

The relation between a chemical reaction rate and temperature was first proposed by Arrhenius:

$$k = A\, e^{-E_a/RT}. \tag{2.5}$$

The constant $A$ is called the frequency factor and is related to the frequency of molecular collisions, $E_a$ is the activation energy or energy required to initiate the reaction, $R$ is the gas constant $R$ and has a value of $8.314 \times 10^{-3}$ kJ mol$^{-1}$ $T^{-1}$, $e$ is the base of the natural logarithm, $T$ is the temperature in degrees Kelvin, and $k$ is the specific reaction rate constant (time$^{-1}$).

Converting Eq. (2.5) to natural logarithmic form gives

$$\ln k = (-E_a/RT) + \ln A. \tag{2.6}$$

By determining the value of $k$ over a moderate range of soil temperatures, the plot of ln $k$ versus $1/T$ yields the activation energy from the slope of a line and the frequency factor from the intercept. Soil chemical reaction rates increase, often very sharply, at low temperatures, with increases in temperature due to increased molecular interactions. It is generally accepted that a temperature coefficient, $Q_{10}$, of approximately 2 over the range 15 to 35°C can be used to describe the relationship between temperature and soil chemical and biochemical processes. That is, a twofold increase or decrease in reaction rate is associated with a shift of 10°C.

But the relationship between temperature and biologically mediated processes is more complicated. While it is expected that the rate of enzyme-catalyzed reactions will increase as temperature increases, at least until some high temperature is reached that causes enzyme inactivation, the increase is not always a factor of 2. Typically enzyme-catalyzed reactions tend to be less affected by temperature changes. Studies have found that a 10°C temperature increase, from 15 to 25°C, can increase soil C and N mineralization rates by up to threefold. And in addition to increasing the specific reaction rate constant, the sizes of the organic matter pools undergoing mineralization are affected by temperature. Thus, the increase in biological activity at higher temperatures is likely due to shifts in microbial community structure.

Though microbial activity at temperatures $<5$°C is slower than at warmer temperatures, it is not negligible and is significantly higher than the $Q_{10}$ relationship developed over the mesophilic range 10 to 20°C would predict. Microbial activity at soil temperatures lower than 0°C has been recorded. Psychrophilic organisms are capable of growth at these low temperatures by adjusting upward the osmotic concentration of their cytoplasmic constituents to permit cell interiors to remain unfrozen. Mineralization activity during cool periods when plants are dormant or soils are barren could play a significant role in overwinter losses of soil nutrients, N in particular.

A generalized temperature response curve for soil microbial activity, assuming soil moisture and aeration are not limiting, is shown in Fig. 2.8.

Rates of reaction increase quite sharply over the mesophilic temperature range from 10 to 25°C, suggesting a selection and/or adaptation of soil microorganisms. Nevertheless different microbial communities are likely active as temperatures change, and while individual species differ in their optimal temperature response, this general activity response to temperature is similar for many organisms.

Very few soils maintain a uniform temperature in their upper layers. Variations may be either seasonal or diurnal. Because of the high specific heat of water, wet

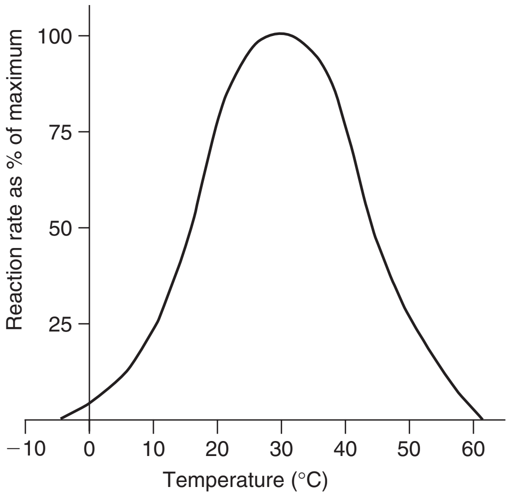



FIGURE 2.8 Generalized soil microbial activity response curve to soil temperature, assuming soil moisture and aeration are not limiting.

soils are less subject to large diurnal temperature fluctuations than are dry soils. Among factors affecting the rate of soil warming, the intensity and reflectance of solar irradiation are critical. The soil's aspect (south- versus north-facing slopes), steepness of slope, degree of shading, and surface cover (vegetation, litter, mulches) determine effective solar irradiation. Given the importance of soil temperature in controlling soil processes, models of energy movement into the surface soil profile have been developed. They are based on physical laws of soil heat transport and thermal diffusivity and include empirical parameters related to the temporal (seasonal) and sinusoidal variations in the diurnal pattern of near-surface air temperatures. The amplitude of the diurnal soil temperature variation is greatly dampened with profile depth.

## SOIL WATER CONTENT

Soil water affects the moisture available to organisms as well as soil aeration status, the nature and amount of soluble materials, the osmotic pressure, and the pH of the soil solution. Water acts physically as an agent of transport by mass flow and as a medium through which reactants diffuse to and from sites of reaction. It acts chemically as a solvent, as a reactant in important chemical and biological reactions, and as a chemical buffer fixing the activity of water in soil solution at about 1. Of special significance in the soil system and to microbial cells in particular is the fact that water adsorbs strongly to itself and to surfaces of soil particles by hydrogen bonding and dipole interactions. The thin layer of adsorbed water remains unfrozen even at temperatures $<0°C$.

Soil water content can be measured on a mass or volume basis. Gravimetric soil water content is the mass of water in the soil, measured as the mass loss in a soil dried at 105°C (oven-dry weight) and is expressed per unit mass of oven-dry soil. Volumetric soil water content is the volume of water per unit volume of soil. Soil water is also described in terms of its potential free energy, based on the concept of matric, osmotic, and gravitational forces affecting water potential. Soil water potential is expressed in units of pascals (Pa) or, more commonly, kilopascals (kPa). Matric forces are attributed to the adhesive or adsorption forces of water attraction to surfaces of mineral and organic particles and to cohesive forces or attraction to itself. Since these forces reduce the free energy status of the water, matric forces reduce the water potential to values less than 0; that is, matric forces result in negative water potentials. Solutes dissolved in soil solution also contribute to a reduction in the free energy of water and give rise to an osmotic potential that too is negative. Combined, the matric and osmotic forces are responsible for the retention of water in soils. They act against gravitational forces tending to draw water downward and out of the soil. Gravitational forces are usually positive.

When the gravitational forces draining water downward are exactly counterbalanced by the matric and osmotic forces holding onto the water, the soil is said to be at field capacity or at its water holding capacity. This will occur after irrigation, after a heavy rainfall, or after spring thaw, which leave the soil saturated and having a soil water potential of 0 kPa. Gravitational forces begin immediately to drain away water in excess of that which can be retained by matric + osmotic forces, leaving the soil after 1–2 days at field capacity. By definition the field capacity for loam and clay loam soils is a soil water potential of –33 kPa, and for sandy soils –10 kPa.

Water retention or soil water content at a given soil water potential is a function of the size of pores present in the soil, or pore size distribution. Soils of different textures have very different water contents even though they have the same water potential (Table 2.2). An important property of water influencing its behavior in soil pores is surface tension. Because of the strong cohesive forces, water has a high surface tension. Based on matric forces and properties of surface tension, the maximum diameter of pores filled with water at a given soil water potential can be estimated using the Young–Laplace equation:

$$\text{Maximum pore diameter retaining water } (\mu\text{m}) = -300/(\text{soil water potential (kPa)}). \qquad (2.7)$$

Those soil pores greater than about 10 μm diameter drain under the influence of gravitation forces, given that the soil water potential at field capacity is –33 kPa.

Soil water potential determines the energy that an organism must expend to obtain water from the soil solution. Generally aerobic microbial activity in soil is optimal at a soil water potential of about –50 kPa and decreases as the soil either becomes wetter and saturated, i.e., waterlogged, or dries (Fig. 2.9).

While plants are at their wilting point when the soil water potential reaches –1500 kPa, relative rates of soil microbial activity can still be quite high.

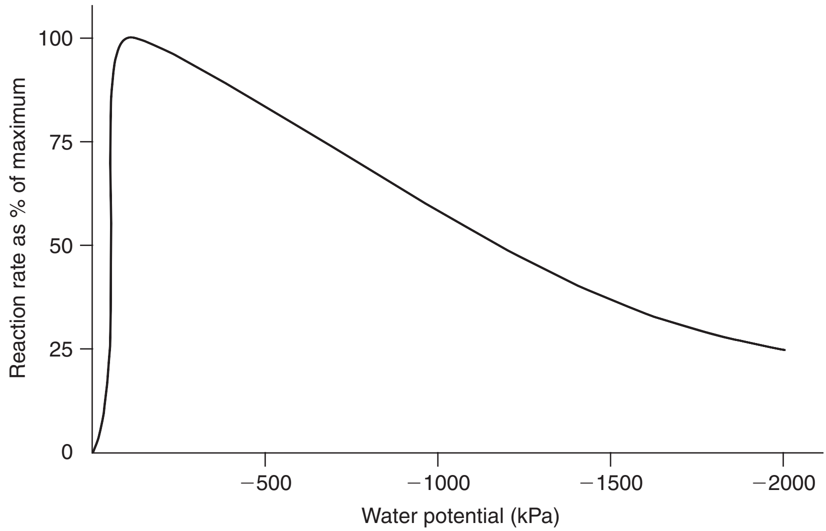



FIGURE 2.9 Generalized aerobic microbial activity response to soil water potential, assuming soil temperature is not limiting.

TABLE 2.5 Ability of Different Organisms to Tolerate Water Stress (from Paul and Clark, 1996)

| kPa | $A_w$ | Organism |
|---|---|---|
| –1500 | 0.99 | *Rhizobium, Nitrosomonas* |
| –1000 | 0.93 | *Clostridium, Mucor* |
| –2500 | 0.83 | *Micrococcus, Penicillium* |
| –6500 | 0.62 | *Xeromyces, Saccharomyces* |

Diffusion of substrates to microorganisms is greatly slowed by drying; however, the relative humidity in the soil remains high. Rapid changes in soil water potential associated with rewetting cause microbes to undergo osmotic shock and induce cell lysis. A flush of activity by the remaining microbes, known as the Birch effect, results from mineralizing the labile cell constituents.

Different microbial communities are likely to be active over the range of water potentials commonly found in soils. A decline in microbial activity at low soil moisture levels can be explained as resulting from limited diffusion of soluble substrates to microbes or to reduced microbial mobility. Fungi are generally considered to be more tolerant of lower soil water potentials, i.e., drier soils, than are bacteria, presumably because soil bacteria are relatively immobile and rely more on diffusion processes for nutrition. Table 2.5 shows differences in the ability of different organisms to tolerate water stress. The nitrifiers, for example, typified by

*Nitrosomonas*, are less tolerant of stress than are the ammonifiers, typified by *Clostridium* and *Penicillium*. Ammonium may accumulate in a droughty soil because the nitrifiers do not have access to ammonium generated at water potentials at which the ammonifiers are still active.

## ENVIRONMENTAL FACTORS, TEMPERATURE AND MOISTURE INTERACTIONS

Soil moisture and temperature are the critical climatic factors regulating soil biological activity. This control is affected by changes in the underlying rates of enzyme-catalyzed reactions. Where water is nonlimiting, biological activity may depend primarily on temperature, and standard Arrhenius theory can be used to predict temperature affects. But as soils dry, moisture is more controlling of biological processes than is temperature. These two environmental influences do not affect microbial activity in linear fashion but display complex, nonlinear, interrelated effects that likely reflect the individual responses of the various microorganisms and their associated enzyme systems.

The interaction of temperature, moisture, and organisms is exemplified by the current discussions about climate change. A hundred years ago, Swedish scientist Svante Arrhenius asked the important question “Is the mean temperature of the ground in any way influenced by the presence of the heat-absorbing gases in the atmosphere?” He went on to become the first person to investigate the effect that doubling atmospheric carbon dioxide would have on global climate. The globe is warming because of increased $CO_2$ in the atmosphere from man’s burning of past SOM depositions (fossil fuels) and from management changes such as cultivation of forested soils as in the Amazon. This in turn is causing the extensive organic deposits in frozen tundra to thaw. The melting allows the vegetation to change as the former tundra becomes boreal in nature. In turn, the trees will result in less light reflection and further increase warming and primary productivity. Will the generally warmer globe result in less overall organic matter as decomposition is increased? Theory such as the Arrhenius equation suggests that the more resistant organic matter compounds with high activation energy should be more decomposable at higher temperatures (Davidson and Janssens, 2006). But as always in soils there are interactions. What is the effect of physical protection by aggregates? Will there be more or fewer soil aggregates in a warmer climate with different vegetation–decomposer interactions, given that aggregates are formed by microorganisms and roots? Many of the environmental constraints affect decomposition reactions by altering organic matter (substrate) concentrations at the site at which all decomposition occurs, that of the enzyme reaction site. We must also consider decomposition rates at the enzyme affinity level; Michaelis–Menten models of enzyme kinetics are covered in Chap. 16 and energy yield in Chap. 9. Changes in microbial community structure (Chap. 8) also will have profound influences. The goal of this chapter is to provide an environmental boundary of the soil habitat and a description of its fundamental physical and chemical properties.

With this as a foundation, later chapters in this volume explore in detail information about organisms, their biochemistry, and their interactions.

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PART

# II

# Soil Biota

# 3

# Physiological and Biochemical Methods for Studying Soil Biota and Their Function

Ellen Kandeler



## INTRODUCTION

Biologically and biochemically mediated processes in soils are fundamental to terrestrial ecosystem function. Members of all trophic levels in ecosystems depend on the soil as a source of nutrients and depend on soil organisms to release and recycle key nutrient elements by decomposing organic residues. These biotic decomposition processes are studied at three levels of resolution (Sinsabaugh *et al.*, 2002). At the molecular level, plant fiber structure and enzymatic characteristics of degradation are investigated. At the organismal level, the focus is on functional gene analyses, regulation of enzyme expression, and growth kinetics, whereas at

TABLE 3.1 Books and Book Chapters of Methods in Soil Microbiology

| Methods in soil microbiology | References |
|---|---|
| Soil microbiology and soil biochemistry | Alef and Nannipieri (1995), Schinner *et al*. (1996), Levin *et al*. (1992), Weaver *et al*. (1994) |
| Fungi | Frankland *et al*. (1991), Newell and Fallon (1991) |
| Actinomycetes | McCarthy and Williams (1991) |
| Digital analysis of soil microorganisms | Wilkinson and Schut (1998) |
| Soil enzymes | Burns and Dick (2002) |
| Tracer techniques ($^{13}C$, $^{14}C$, $^{11}C$, $^{15}N$) | Schimel (1993), Boutton and Yamasaki (1996), Coleman and Fry (1991), Knowles and Blackburn (1993) |
| Gross nitrogen fluxes ($^{15}N$ pool dilution) | Murphy *et al*. (2003) |
| Soil biological processes and soil organisms | Robertson *et al.* (1999) |

the community level, research concentrates on metabolism, microbial succession, and competition between microbial and faunal communities. These three levels must be integrated to fully understand microbial functions in soils (Sinsabaugh *et al.*, 2002). The function of soil biota is investigated by a range of methods focusing either on broad physiological properties (e.g., soil respiration, N-mineralization) or on specific enzymatic reactions carried out by soil microorganisms (e.g., ammonia monooxygenase of nitrifiers). The activity of approximately 100 enzymes has been identified in soils (Tabatabai and Dick, 2002). A challenge for the future is to localize these enzymes in soils and relate their activity to soil processes at higher levels of resolution.

This chapter focuses primarily on the more important biochemical and physiological methods applied in soil microbiology and soil biochemistry today. Biochemical techniques are used to determine the distribution and diversity of soil microorganisms, whereas physiological methods are used to understand the physiology of single cells, the activity of soil microbial communities, and biogeochemical cycling at the ecosystem level. Faunal abundance and activity are discussed in Chap. 7. Table 3.1 provides references for more detailed studies of biochemical and physiological methods used to study soil microorganisms.

## SCALE OF INVESTIGATIONS AND COLLECTION OF SAMPLES

Before starting any analysis in soil microbiology, it is important to select an adequate experimental design and sampling strategy. Pico- and nanoscale investigations are used to reveal the structure and chemical composition of organic substances and microorganisms as well as to investigate the interactions between the biota and humic substances. These fine-scale approaches can identify organisms,

TABLE 3.2 Physical, Chemical, and Biological Properties That Help to Interpret Data on the Function and Abundance of Soil Biota

| Physical and chemical soil properties | | Biological soil properties |
|---|---|---|
| Topography | Particle size and type | Plant cover and productivity |
| Parent material | $CO_2$ and $O_2$ status | Vegetation history |
| Soil type, soil pH | Bulk density | Abundance of soil animals |
| Moisture status | Temperature: range and variation | Microbial biomass |
| Water infiltration | Rainfall: amount and distribution | Organic matter inputs and roots present |

unravel their relationships, determine their numbers, and be used to measure the rates of physiological processes. Such results boost our understanding of chemical and biological processes and structures at larger scales. Microscale investigations concentrate either on soil aggregates or on microhabitats characterized by high turnover of organic materials (e.g., the rhizosphere, drilosphere, and soil–litter interface). High-activity areas are heterogeneously distributed within the soil matrix. Hot spots of activity may make up less than 10% of the total soil volume, yet may represent more than 90% of the total biological activity (Beare *et al.*, 1995). Up-scaling data from the microscale to the plot or regional scale remains difficult because spatial distribution patterns are still largely unknown.

Sampling at the plot scale is the most common strategy used for soil chemical and biological studies. A representative number of soil samples is taken from the study site and either combined to make a composite sample or treated as individual, spatially explicit samples. Typically, a series of random samples is taken across representative areas that are described by uniform soil type, soil texture, and habitat characteristics. Samples of agricultural soils are often taken from specific soil depths (e.g., 0–20 or 0–30 cm); samples of forest soils are taken from specific soil horizons (e.g., litter horizon, A horizon). Descriptions of sampling time, frequency, and intensity as well as preparation, archiving, and quality control are given by Robertson *et al.* (1999). Soil microbiological data obtained from soil samples become more informative if supplemented by information on the soil physical, chemical, and biotic factors (Table 3.2).

Approaches to sampling must take into account spatial distribution of the soil biota, which depends highly on the organisms studied and the characteristics of the study area (Table 3.3). When topography and soil chemical and physical properties are relatively uniform, spatial patterns of soil biota are structured primarily by plants (plant size, growth form, and spacing). Therefore, simple *a priori* sampling designs are often inappropriate. A nested spatial sampling design is useful to explore spatial aggregation among a range of scales. For patch size estimation and mapping at a particular scale, the spatial sampling design can be optimized using simulations. To increase the statistical power for hypothesis testing in belowground field experiments and monitoring programs, exploratory spatial sampling and

TABLE 3.3 The Relationship of Soil Microbial Properties to Sampling Scale, Taxonomic Resolution, and Ecosystem[a]

| Property | Ecosystem studied | Main factors causing microbial spatial pattern | Patch size (m) | Distance sampled (m) | |
|---|---|---|---|---|---|
| | | | | Min | Max |
| Microbial biomass | Arable and pasture | Land use and $C_{org}$ | 250 | 50 | 1500 |
| | Arable | Topography, $C_{org}$ and moisture | 21 | 5 | 1200 |
| | Spruce–birch | Tree species patch size | 7 | 2 | 20 |
| | Spruce | Tree size and spacing | 1.00 | 0.10 | 18 |
| FAME profiles[b] | Arable | Corn rhizosphere and plant spacing | 0.05–0.97 | 0.02 | 80 |
| $NH_4^+$ oxidizers | Arable | Soil pores, aggregates, and fine roots | 0.004 | 0.001 | 0.09 |

Patch size is estimated by the range parameter of the semivariogram model.
[a]Adapted from Ettema and Wardle (2002).
[b]FAME, fatty acid methyl ester profiles characterizing biomass of specific microbial isolates.

geostatistical analysis can be used to design a hot-spot stratified sampling scheme (Robertson, 1994; Klironomos *et al.*, 1999).

Knowledge of the spatial dependency of soil biota attributes helps to interpret their ecological meaning at the ecosystem scale. Biochemical processes in the soil are dynamic, leading to variation in both space and time. Landscape-scale analyses by geostatistical methods are useful tools for identifying and explaining spatial relationships between soil biochemical processes and site properties. Further model improvements, however, should focus on identifying and mapping time–space patterns using modern approaches such as fuzzy classification and geostatistical interpolation.

## STORAGE AND PRETREATMENT OF SAMPLES

Biological analyses should be performed as soon as possible after soil sampling to minimize the effects of storage on soil microbial communities. Moist soil can be stored for up to 3 weeks at 4°C when samples cannot be processed immediately. If longer storage periods are necessary, the samples taken to measure most soil biochemical properties (soil microbial biomass, enzyme activities, etc.) can be stored at −20°C; the soil is then allowed to thaw at 4°C for about 2 days before analysis. The soil disturbance associated with sampling may itself trigger changes in the soil population during the storage interval. Observations on stored samples may not be representative of the undisturbed field soil. If samples are stored, care should be taken to ensure that samples do not dry out and that anaerobic conditions

do not develop. Soil samples are often sieved through a 2-mm mesh screen to remove stones, roots, and debris prior to analysis. Wet soil samples have to be either sieved through a 5-mm mesh or gently predried before using the 2-mm mesh sieve.

## MICROBIAL BIOMASS

### CHLOROFORM FUMIGATION INCUBATION AND EXTRACTION METHODS

Microbial biomass is measured to give an indication of the response of soil microbiota to management, environmental change, site disturbance, and soil pollution. Two different approaches are both based on $CO_2$ evolution. The chloroform fumigation incubation (CFI) method (Jenkinson and Powlson, 1976) exposes moist soil to ethanol-free chloroform for 24 h to kill the indigenous microorganisms. After removal of the fumigant, a flush of mineralized $CO_2$ and $NH_4^+$ is released during a 10-day incubation. This flush is caused by soil microorganisms that have survived the fumigation (as spores or cysts) and use cell lysates as an available C and energy source. The released $CO_2$ is trapped in an alkaline solution and is quantified by titration. Alternatively, the $CO_2$ that accumulates in the headspace of the sample containers is measured by gas chromatography. An assay of nonfumigated soil serves as a control. The amount of microbial biomass C is calculated as

$$\text{biomass C} = (F_C - Uf_C)/K_C,$$

where biomass C is the amount of carbon trapped in the microbial biomass, $F_C$ is the $CO_2$ produced by the fumigated soil, $Uf_C$ is the $CO_2$ produced by the nonfumigated soil sample, and $K_C$ is the fraction of the biomass C mineralized to $CO_2$. The $K_C$ value is a constant representative of the cell utilization efficiency of the fumigation procedure. This efficiency is considered to be about 40–45% for many soils (e.g., a constant of 0.41–0.45). Deviations of this range are found for subsurface and tropical soils. The $K_C$ factor of soil samples can be estimated by measuring the $^{14}CO_2$ release of soil microorganisms isolated from different soils that use radiolabeled bacterial cells as substrates.

The chloroform fumigation extraction (CFE) method involves the extraction and quantification of microbial constituents (C, N, S, and P) immediately following $CHCl_3$ fumigation of the soil (Brookes *et al.*, 1985). The efficiency of soil microbial biomass extraction has to be taken into account. A $K_{ec}$ factor of 0.45 is recommended for agricultural soils (Joergensen, 1996), and the $K_{ec}$ factor of soils from other environments (subsurface soils, peat soils, etc.) should be experimentally derived. The CFE method can be applied to a wide range of soils. Soils containing large amounts of living roots require a preextraction procedure of roots because these cells are also affected by the fumigation procedure.

## SUBSTRATE-INDUCED RESPIRATION

The substrate-induced respiration (SIR) method estimates the amount of C held in living, heterotrophic microorganisms by measuring the initial respiration after the addition of an available substrate (Anderson and Domsch, 1978). In general, soil samples are placed into airtight containers and then amended with glucose. Evolved $CO_2$ is followed for several hours (no proliferation of microorganisms should occur under these conditions). The initial respiratory response is proportional to the amount of microbial C present in the soil sample. Applying the following conversion factor, derived from the calibration of substrate-induced respiration to the chloroform fumigation incubation technique, results can be converted to milligrams of biomass C,

$$y = 40.04x + 0.37,$$

where $y$ is biomass C (mg 100 g$^{-1}$ dry wt soil) and $x$ is the respiration rate (ml $CO_2$ 100 g$^{-1}$ soil h$^{-1}$). Respired $CO_2$ can be measured by use of an alkali trap followed by titration or by GC analysis of the headspace gas. The optimum concentration of glucose leading to the maximal initial release of $CO_2$ has to be independently determined for each soil type and should be applied to that soil to standardize the SIR method between different soil types. The SIR method using titrimetric measurement of $CO_2$ is frequently applied because it is simple, fast, and inexpensive. A disadvantage of the static systems with alkaline absorption of evolved $CO_2$ is that the $O_2$ partial pressure may change, causing overestimations in neutral or alkaline soils. Nevertheless, most versions of the three methods for estimating microbial biomass (CFI, CFE, and SIR) gave identical ranking from a range of 20 arable and forest sites in an interlaboratory comparison (Beck *et al.*, 1997).

Using selective antibiotics, the SIR approach can also be used to measure the relative biomass of fungi and bacteria in the soil microbial community. Glucose-induced respiration is determined for fungi in the presence of streptomycin, which inhibits prokaryotes, and for bacteria in the presence of cycloheximide (actidione), which inhibits eukaryotes. An automated infrared gas analyzer system is used to continuously measure $CO_2$ produced, and a computer program is used to calculate the bacterial/fungal respiration based on the following criteria: (1) proof of no unselective inhibition and (2) proof of no shifts in the biosynthesis rates of bacteria and fungi in favor of one group (Bååth and Anderson, 2003).

## ISOTOPIC COMPOSITION OF MICROBIAL BIOMASS

Determining the isotopic composition of microbial biomass C is a further important tool for studying soil microbial ecology and the decomposition and immobilization of soil organic C. The use of the CFI method is restricted to the isotopes $^{14}C$, $^{13}C$, and $^{15}N$, whereas the CFE method can be used with a larger range of isotopes (i.e., $^{14}C$, $^{13}C$, $^{15}N$, $^{32}P$, and $^{35}S$). Recently, $^{13}C$ studies have

gained increasing interest due to the improved sensitivity of $^{13}C$ measurements and due to its nonradioactive nature (in contrast to $^{14}C$ studies). Since the tissues of $C_3$ plants (e.g., wheat) and $C_4$ plants (e.g., maize) differ in their natural abundance of $^{13}C$, these plant materials have a natural label that can be used for decomposition studies in both microcosm and field studies. $^{13}C/^{12}C$ determinations are performed with an offline sample preparation technique combined with isotope analysis by a dual-inlet IRMS (isotope ratio mass spectrometer) or an online analysis using an element analyzer connected to an isotope ratio mass spectrometer. A new method is based on the UV-catalyzed liquid oxidation of fumigated and nonfumigated soil extracts combined with trapping of the released $CO_2$ in liquid $N_2$; $\delta^{13}CO_2$–C is subsequently determined with a gas chromatograph connected to an IRMS (Potthoff *et al.*, 2003). The $^{13}C$ analysis can also be done using an automated continuous-flow IRMS.

## SIGNATURE MOLECULES AS A MEASURE OF MICROBIAL BIOMASS AND MICROBIAL COMMUNITY STRUCTURE

Various cellular constituents can be used to estimate microbial biomass and subdivide community members into broad groups. These signature molecules include adenosine triphosphate (ATP), microbial membrane components, and respiratory quinones. Each type of molecule differs in its level of resolution. The amount of ATP extracted from soil gives a measure of the energy charge of all soil biota, whereas ergosterol, a component of fungal cell walls, is attributed only to the fungal biomass. Signature molecules such as phospholipid fatty acids or respiratory quinones are used as indicators of the microbial community's structural diversity. A prerequisite for the use of biochemical compounds as signature molecules is that they are unstable outside the cell, because the compound extracted from soil should represent living organisms only.

### ATP AS A MEASURE OF ACTIVE MICROBIAL BIOMASS

All biosynthetic and catabolic reactions within cells require the participation of ATP. ATP is sensitive to phosphatases and does not persist in soil in a free state. It should be ideal for determining the amount or activity of life within soil, sediment, or aquatic systems. The substrate luciferin, an aromatic N- and S-containing molecule, reacts with ATP and luciferase in the presence of $Mg^{2+}$ to yield an enzyme–luciferin–adenosine monophosphate intermediate. This, in the presence of $O_2$, breaks down to produce free adenosine monophosphate, inorganic P, and light. The light emitted is measured by a photometer or scintillation counter and plotted against ATP content to form a standard curve. If pure luciferase and

luciferin rather than firefly tails are used, the light output is extended and constant. Useful extracting reagents include combinations of anions (phosphate) and cations that adequately lyse cells, inhibit adsorption of the ATP to soil surfaces, and inhibit the numerous soil ATPases. Most extraction reagents inhibit the luciferase reaction to some extent; therefore, the solution is diluted as much as possible before measurement. A mixture consisting of phosphoric acid, urea, dimethyl sulfoxide, adenosine, EDTA, and polyoxyethylene-10-laryl ether is now often used. Total adenylates (ATP, ADP, and AMP) can be measured after extraction with dimethyl sulfoxide and a 0.01 M $Na_3PO_4$/0.02 M EDTA buffer by the HPLC (high-performance liquid chromatography) method. During microbial growth, the C:ATP ratio can vary from 1000:1 to 40:1. In the resting state, the ATP:C:N:P:S ratios are usually 1:250:40:9:2.6. ATP measures of both biomass and activity are influenced by the soil P content. This method can be used most successfully to characterize soils whose microbial population is mainly in the resting state at excess or constant P levels. As such, it is a rapid and sensitive technique. The measurement of ATP content relative to AMP and ADP plus ATP, $(ATP + 0.5 \times ADP)/(AMP + ADP + ATP)$, gives a measure of the adenylate energy charge of the soil biota.

## MICROBIAL MEMBRANE COMPONENTS AND FATTY ACIDS

Lipids occur in microbial membranes and as storage products. More than 1000 different individual lipids have been identified. Lipids can be extracted from the soil biota with a one-phase chloroform methane extraction. Separation of the extracted lipids on silicic acid columns yields neutral lipids, glycolipids, and polar lipids. The neutral lipids can be further separated by HPLC, derivatization, and gas chromatography to yield quinones, sterols, and triglycerides. Glycolipids, on hydrolysis and derivatization followed by gas chromatography, yield poly-β-hydroxybutyrate. The mixture of polymers can be analyzed to determine the nutritional status of bacteria such as those associated with plant roots. Polar lipids are separated by hydrolysis, derivatization, and gas chromatography to yield phospholipid phosphate, phospholipid glycerol, phospholipid fatty acids, and ether lipids (Table 3.4). Concentration of lipids in soil apart from those of microorganisms is low; the amount in organisms from different taxa is quite variable. It is thus difficult to relate the quantities extracted to biomass. Saponification and esterification after extraction followed by gas chromatography or HPLC produce a broad spectrum of the free plus bound cell fatty acids. This is known as fatty acid methyl esters and is useful in general community diversity analyses.

Phospholipids are found in the membranes of all living cells, but not in storage products, and are rapidly turned over upon cell death. They are therefore excellent signature molecules. The phospholipid fatty acid (PLFA) technique has been used to elucidate different strategies employed by microorganisms to adapt to changed environmental conditions under a range of soil types, management practices, climatic origins, and perturbations. PLFAs are extracted in single-phase solvent extractions

TABLE 3.4 Representative Fatty Acids Found in Microorganisms[a,b]

| Fatty acid | Example | Designation | Name |
|---|---|---|---|
| Saturated | $CH_3(CH_2)_{14}COOH$ | 16:0 | Palmitate |
| Monounsaturated | $CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$ | 16:1ω7 | Palmitoleate |
| Polyunsaturated | $CH_3(CH_2)_5CH{=}CHCH_2CH{=}CH(CH_2)_6COOH$ | 18:2ω7 | Linoleate |
| Iso | $CH_3–C(CH_2)_{12}COOH$ with $CH_3$ branch | i16:0 | — |
| Anteiso | $CH_3CH_2CH(CH_2)_{11}COOH$ with $CH_3$ branch | a16:0 | — |

[a]Fatty acids are described as follows. The number of carbons in the chain is followed by a colon, then by the number of unsaturations. ω precedes the number of C atoms between the terminal double bond and the methyl end of the molecule. c, *cis* (most common form, omitted in most cases); t, *trans*; i, iso methyl branching (second C from the methyl end); a, anteiso methyl branching (third C from the methyl end); Me, follows position of methyl branching; cy, cyclopropane ring.

[b]Adapted from Vestal and White (1989).

and can be analyzed by: (1) colorimetric analysis of the phosphate after hydrolysis, (2) colorimetric analysis or gas chromatography (GC) after esterification, (3) capillary GC, and (4) GC–mass spectrometry or triple-quadruple mass spectrometry. The mass profile on mass spectrometry yields information on phospholipid classes present and on their relative intensities. Fragmentation spectra can provide the empirical formulas. The phosphatidic mass profiles can be compared by constructing a dendrogram to determine similarity indices of phospholipids from isolated organisms. The type of phospholipid fatty acid group also supplies information; bacteria contain odd-chain methyl-branched and cyclopropane fatty acids.

The odd-number and branched-chain fatty acids are produced by gram-positive ($G^+$) bacteria, whereas the even-number, straight-chain and cyclopropyl fatty acids tend to be derived from $G^-$ bacteria (Table 3.5). The straight-chain fatty acids, although of limited taxonomic value, can be used as indicators of microbial biomass. Unsaturation is associated with anaerobiosis, e.g., 18:1; 11C is found in anaerobic bacteria as well as in most $G^-$ aerobes. The fatty acids 18:2ω6 account for 43% of the total fatty acids of 47 species of soil fungi. The fatty acids of arbuscular mycorrhizal fungi (AMF) offer special opportunities for identification and quantification; 16:1ω5 is found in the AMF genus *Glomus* and 20:1ω9 in the *Gigaspora* species. These fatty acids make it possible to differentiate non-AMF and provide an infection index for plant roots. $^{14}C$ and $^{13}C$ labeling and measurement of the tracer in fatty acids such as those in the AMF allow *in situ* determinations of turnover rates of the mycorrhizal symbionts by GC/MS. The ratios of cyclopropyl/monoenoic precursors and total saturated/total monounsaturated fatty acids are applied as indicators of microbial stress in soils (Fierer *et al.*, 2003). Recently, the fatty acid composition of soil animals was also used as indicator of animal diets in belowground systems (Ruess *et al.*, 2004).

TABLE 3.5 Marker Fatty Acids in the Phospholipid Fraction of Several Groups of Organisms

| Fatty acid | Organisms |
|---|---|
| 16:1ω9, 15:0, i15:0 | Eubacteria in general, cyanobacteria, actinomycetes |
| Cy15:1 | Clostridia |
| 16:0, 18:3ω3 | Fungi |
| i16:0 | Gram-positive bacteria |
| 16:1ω5 | Cyanobacteria, AM fungi (e.g., *Glomus*) |
| 16:1ω7, 16:1ω7t | Eubacterial aerobes |
| 16:1ω13t | Green algae |
| cy17:0, cy19:0 | Eubacterial anaerobes, gram-negative bacteria |
| 17:1ω6, i17:1ω7 | Sulfate-reducing eubacteria, actinomycetes |
| 18:1ω7 | Eubacterial aerobes, gram-negative bacteria |
| 18:1ω9 | Fungi, green algae, higher plants, gram-positive bacteria |
| 18:1ω11, 26:0 | Higher plants |
| 18:2ω6 | Eukaryotes, cyanobacteria, fungi |
| 18:3ω3, 18:3ω6 | Fungi, green algae, higher plants |
| 20:1ω9 | AM fungi (e.g., *Gigasporea rosea*) |
| 20:3ω6, 20:4ω6 | Protozoa |
| 20:5, 22:6 | Barophyllica, psychrophilic eubacteria |

While PLFA profiling is a well-established method in soil ecology, phospholipid ether lipid (PLEL) analyses for the characterization of Archaea is a rather new approach (Gattinger *et al.*, 2003). PLEL-derived isoprenoid side chains are measured by GC/MS and provide a broad picture of the archaeal community in a mixed soil extract, because lipids identified in isolates belonging to the Subkingdom Eury- and Crenarchaeota are covered. Monomethyl-branched alkanes dominate and account for 43% of the total identified ether-linked hydrocarbons, followed by straight-chain (unbranched) and isoprenoid hydrocarbons, which account for 34.6 and 15.5%, respectively.

## RESPIRATORY QUINONES AS A MEASURE OF STRUCTURAL DIVERSITY

The quinone profile, which is represented as a molar fraction of each quinone type in a soil, is a simple and useful tool to analyze population dynamics in soils; the total amount of quinones can be used as an indicator of microbial biomass (Fujie *et al.*, 1998). Quinones are essential components in the electron transport systems of most organisms and are present in the membranes of mitochondria and chloroplasts. Isoprenoid quinones are chemically composed of benzoquinone (or naphthoquinone) and an isoprenoid side chain (Fig. 3.1). There are two major groups of quinones in soils: ubiquinones (1-methyl-2-isoprenyl-3,4-dimethoxyparabenzoquinone) and menaquinones (1-isoprenyl-2-methylnaphthoquinone). The nomenclature of bacterial quinones is as follows: the abbreviation for the type of quinone (ubiquinone, Q; menaquinone, MK) followed by a dash and the number of isoprene units in its side chain and the number of hydrogen atoms in the

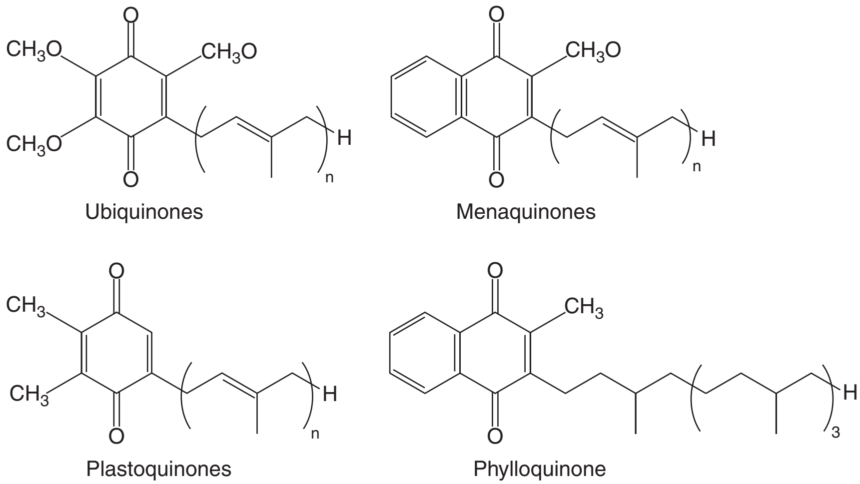



FIGURE 3.1 Representative isoprenoid quinones observed in microorganisms (with permission from Katayama and Fujie, 2000).

hydrogenation of double bonds in the side chain (in parentheses). For example, UQ-10 stands for a ubiquinone with 10 isoprene units in its side chain and MK-8(H2) represents menaquinone with 8 isoprenoid units, of which one double bond is hydrogenated with 2 atoms of hydrogen. Most microorganisms contain only one type of quinone as their major quinone, which remains unchanged by physiological conditions. The half-lives of those quinones released by dead soil microorganisms are very short (in the range of several days). The diversity of quinone molecules can be interpreted directly as an indicator of microbial diversity. About 15–25 types of quinones are detected in most soils. The distribution of the most important marker quinones within several groups of organisms is given in Table 3.6. Many $G^-$ bacteria contain Q-8, Q-9, and Q-10. $G^-$ bacteria containing Q-8 are mostly classified in the $\beta$ subgroup of the proteobacteria and some in the $\gamma$ subgroup. $G^-$ bacteria in the *Cytophaga–Flavobacterium* complex contain MK-6 and MK-7. $G^+$ bacteria contain only menaquinones. The high mole ratios of menaquinones to ubiquinones in soils support earlier results using culturing and isolation techniques that showed that $G^+$ bacteria dominate in soil. As $G^+$ bacteria have a wide variety of menaquinones, the sensitivity of quinone profiles to changes in the microbial community — especially with respect to $G^+$ bacteria in soil — is higher than the sensitivity of PLFA profiles, which detect $G^+$ bacteria based on the presence of only one signature compound (branched ester-linked fatty acids).

## ERGOSTEROL AS A MEASURE OF FUNGAL BIOMASS

This predominant sterol of most fungi does not occur in plants and has been used to measure invasions of pathogenic fungi into plants. The ergosterol content

TABLE 3.6 Marker Quinones of Several Groups of Organisms[a]

| Quinone | Organisms |
|---|---|
| Ubiquinones | |
| Q-8 | Gram-negative bacteria, mainly β-proteobacteria (*Acinetobacter*, *Alicaligenes*, *Enterobacteriaceae*) |
| Q-9 | Gram-negative bacteria, mainly γ-proteobacteria, fungi |
| Q-10 | Gram-negative bacteria |
| Q-10(H2) | Fungi |
| Q-10(H4) | Fungi (*Penicillium*) |
| Menaquinones | |
| MK-6 | Gram-negative bacteria (*Cytophaga–Flavobacterium*), δ- and ε-proteobacteria |
| MK-7 | Gram-positive (low G + C contents, *Bacillus*) and gram-negative bacteria (*Cytophaga–Flavobacterium*) |
| MK-8 | Gram-negative bacteria (*Proteus*, *Enterobacter*) |
| MK-10(H6), MK-10(H8) | Gram-positive bacteria, *Streptomycineae*, *Micromonosporinae* in the Class of *Actinobacteria* |
| MK-10, MK-11, MK-12 | Gram-positive bacteria (*Agrococcus*, *Aureobacterium*, *Microbacterium*) |

[a]Adapted from Fujie *et al.* (1998), Katayama and Fujie (2000), and Hu *et al.* (2001).

of soils indicates the extent of fungal membranes as well as fungal and ectomycorrhizal biomass. Ergosterol is extracted by methanol and detected using high-performance liquid chromatography with a UV detector. Since chromatographic coelution might be a problem, reversed-phase liquid chromatography with positive-ion atmospheric pressure chemical ionization tandem mass spectrometry can be used for full quantification and confirmation of ergosterol (Verma *et al.*, 2002). The ergosterol content varies from 0.75 to 12.9 $\mu g\ g^{-1}$ soil in arable, grassland, and forest soils. These values correspond to 5 to 31 mg ergosterol $g^{-1}$ fungal dry weight depending on species and growth conditions. The ratio of ergosterol to microbial biomass C is used as an index for fungal biomass to the total soil microbial biomass. Shifts in microbial community structure due to soil contamination or changes in vegetation can be detected using the ergosterol to microbial biomass C ratio. In addition, the content of coprostanol, which is a sterol present after sewage sludge disposal and contamination by municipal wastes, is a useful marker of human fecal matter contamination of soils.

## LIPOPOLYSACCHARIDES, GLYCOPROTEINS, AND CELL WALLS

The outer cell membrane of $G^-$ bacteria contains unique lipopolysaccharide polymers that can be used as biomarkers. The peptidoglycan of bacterial cell walls contains *N*-acetylmuramic acid and diaminopimelic acid. Chitin, a polymer of *N*-acetylglucosamine, is found in many fungi. This compound is also present in the exoskeleton of invertebrates. The usefulness of chitin assays is limited by the need

for acid hydrolysis prior to analysis, the variability in the amount present, the presence of chitin in a wide variety of other organisms, and its accumulation in nonbiological soil components.

Arbuscular mycorrhizal fungi contain a recalcitrant AMF-specific glycoprotein, glomalin, in their cell walls, which can be quantified operationally in soils as glomalin-related soil protein (Driver *et al.*, 2005). An indirect immunofluorescence assay is described by Wright (2000) to detect glomalin on AMF hyphae attached to roots, in roots, on hyphae, and on the surfaces of soil aggregates. Since glomalin is a recalcitrant compound, this glycoprotein cannot be used as a signature molecule for living AMF.

### GROWTH RATES FROM SIGNATURE MOLECULES

Growth and turnover rates can be determined by incorporating tracer isotopes of C into precursors of cytoplasmic constituents, membranes, or cell wall components. The increase in the tracer over short periods yields estimates of growth. For example, a technique to estimate relative fungal growth rates was based on the addition of [$^{14}$C]acetate to a soil slurry and measurement of the subsequent uptake and incorporation of the labeled acetate into the fungus-specific sterol, ergosterol (Newell and Fallon, 1991). The specificity of this incorporation was shown by using fungal and bacterial inhibitors. Incorporation rates were linear up to 18 h after the acetate was added, but absolute growth rates could not be calculated due to the uncertainty of conversion factors and problems associated with saturation of the incorporation of the added acetate. Similar techniques have also been developed for C isotopes in microbial lipids. Fatty acids may also be used in combination with their role as biomarkers to monitor the C flux in a bacterial community by measuring the ratios of their C isotopes. Before fatty acids can be used as chemotaxonomic markers and as indicators of substrate use in microbial communities, calibration studies on the degree and strain specificity of isotopic $^{13}$C fractionation with regard to the growth substrate are necessary.

## PHYSIOLOGICAL ANALYSES

A wide array of physiologically based soil processes are known, e.g., decomposition, ammonification, nitrification, denitrification, $N_2$ fixation, P mineralization, and S transformations. These processes are described in detail in later chapters. Here I cover primarily culture-based studies, techniques to measure respiration, N-mineralization, and enzyme measurements.

### CULTURE-BASED STUDIES

Culturing a soil organism involves transferring its propagules to a nutrient medium conducive to its growth. Culture-based techniques allow specific soil microorganisms to be isolated from a wide range of soils. Culturing techniques

are selective and designed to detect microorganisms with particular growth forms or biochemical capabilities. Bacterial cells from surfaces of soil particles and aggregates are liberated in sterile water, Windogradsky's solution, or physiological saline (0.85% NaCl). After extraction soil suspensions are diluted to an appropriate concentration. The degree of dilution required is related to the initial number of organisms (or propagules) in the soil. Serial dilutions, usually 1:10, stepwise, are made, beginning with a known weight of wet soil. Aggregates are broken by brief mixing in a Waring blender, often in the presence of a dispersing agent such as $Na_4P_2O_7$, prior to performing serial dilutions. Replicate 0.1-ml portions of appropriate suspensions are transferred to solid or liquid medium in which individual propagules develop into visible growth. After appropriate incubation, single colonies on solid media are counted, and each colony is equated with a single propagule in the soil suspension (colony-forming unit).

The plate count of bacteria in soil, water, and sediment usually represents 1 to 5% of the number determined by direct microscopy, leading to many discussions concerning viable but nonculturable bacteria in nature (Kjelleberg, 1993) and the usefulness of this method for quantitative soil microbiology. Plate count methods are not suitable for enumerating fungal populations or densities because both individual spores and fragments of hyphae develop into colonies that are counted. Due to these strong limitations, many researchers doubt whether plate counts can be used for quantification of soil microbiota.

Population densities of various groups of bacteria can be estimated by the most probable number (MPN) technique. This method uses a liquid medium to support growth of soil microorganisms. The resulting dilution count is based on determining the highest soil dilution that will still provide visible growth in a suitable medium. An actual count of single cells or colonies is not necessary. Inoculating replicate tubes (10 usually, 5 minimum) from each of three successive serial dilutions at the estimated extinction boundary for growth enables resultant visual growth to be converted to numbers within statistical limits of reliability (Gerhardt *et al.*, 1994). Computer software has been developed to determine MPN and related confidence limits as well as to correct for biases (Klee, 1993). MPN methodology is useful because it allows a bacterial population to be estimated based on process-related attributes such as nitrification by *Nitrosomonas* and *Nitrobacter* spp., denitrification by denitrifiers, or nitrogen fixation by free-living aerobic and microaerophilic $N_2$-fixing bacteria. The MPN technique requires an appropriate choice of growth medium and accurate serial dilutions to obtain quantitative data. In general, the results are usually less precise than those obtained with direct plating methods and suffer from similar biases due to the ability of cells to grow in artificial media.

## ISOLATION AND CHARACTERIZATION OF SPECIFIC ORGANISMS

Specific, culturable organisms are usually isolated from soil by using liquid or solid substrates. These can involve very general substrates from which individual colonies are picked for further characterization. Also useful are media containing

inhibitors such as chloramphenicol, tetracycline, and streptomycin, which inhibit protein synthesis by binding to the 50S ribosomal subunit, or nalidixic acid, which inhibits DNA synthesis of $G^-$ bacteria. Specific growth conditions include high or low pH, aerobic or anaerobic, or high salinity. Plants are used to identify and enrich specific soil populations. Growing compatible legumes in test soils makes it possible to isolate and identify rhizobia from root nodules. Root or leaf pathogens and mycorrhizal fungi are often similarly enriched and identified. Isolations can be performed without growth media by microscopic examination combined with micromanipulators or optical laser tweezers, which can separate single cells, spores, or hyphal fragments.

Genetic markers that are incorporated into isolated organisms before soil reinoculation include antibiotic resistance, the lux operon for light emission, and genes that code for specific enzyme activities. Resistance to most currently used antibiotics is usually carried on a plasmid (see Chap. 5). The use of an appropriate vector makes it possible to transfer a plasmid containing an antibiotic resistance gene to soil isolates. These isolates can then be reintroduced into soil and recovered by placing soil dilutions on the appropriate medium containing that antibiotic. Only organisms carrying the antibiotic resistance gene(s) will be capable of growth.

Automated, multisubstrate approaches have been used as metabolic fingerprinting system. For example, the Biolog system makes it possible to test growth reactions of many isolates on a broad range of sole C substrate and to analyze the community level respiratory response of culturable organisms in a soil sample. This, combined with the use of a broad range of known organisms and mathematical clustering techniques, allows researchers to characterize unknown organisms by sorting them into affinity types occurring in a particular habitat. Because of their generally oxidative and mycelial nature, it is not possible to characterize fungi using substrate-utilizing clustering analyses. A community-level physiological approach is based on soil dilutions into microtiter plates in which redoxdyes are added to multiple, single C substrates. This approach bypasses the need to work with isolated culturable organisms but still requires growth (reduction) on the appropriate substrate. Multiple substrate fingerprinting has limitations (like measuring an enzyme activity of a growing microbial population or the selectivity of the microbial response) and is controversial in soil microbiology.

## SOIL ORGANIC MATTER DECOMPOSITION AND RESPIRATION

Heterotrophic microbial communities oxidize naturally occurring organic material such as carbohydrates according to the generalized equation

$$CH_2O + O_2 \rightarrow CO_2 + H_2O + \text{intermediates} + \text{cellular material} + \text{energy.}$$

Under anaerobic conditions, the most common heterotrophic metabolic pathway is that of fermentation, which in its simplest form is described as

$$C_6H_{12}O_6 \rightarrow 2\ CH_3CH_2OH + 2\ CO_2 + \text{intermediates} \\ + \text{cellular material} + \text{energy.}$$

Measuring microbial activity is complex under anaerobic conditions. Fermentation products and $CH_4$ produced within anaerobic microsites can diffuse to aerobic areas, where oxidation to $CO_2$ and $H_2O$ can occur. Microbial respiration is determined by measuring either the release of $CO_2$ or the uptake of $O_2$. Because the atmospheric $CO_2$ concentration is only 0.036%, versus 20% for $O_2$, measurements of $CO_2$ production are more sensitive than those for $O_2$. One method of $CO_2$ measurement involves aeration trains; here, NaOH is used to trap evolved $CO_2$ in an airstream from which $CO_2$ is removed before the air is exposed to the soil sample. The reaction occurs as follows:

$$2\ NaOH + CO_2 \rightarrow Na_2CO_3 + H_2O.$$

Before titration, $BaCl_2$ or $SrCl_2$ is added to precipitate the $CO_3^{2-}$ as $BaCO_3$ or $SrCO_3$, and excess NaOH is backtitrated with acid. The use of carbonic anhydrase and a double endpoint titration provides greater accuracy when $CO_2$ concentrations are low. In the laboratory, NaOH containers placed in sealed jars are convenient and effective for $CO_2$ absorption. The jar must be opened at intervals so that the $O_2$ concentration does not drop below 10%. After incubation, the NaOH trap is titrated as described above or the electric conductivity of the alkali trap is measured to calculate $CO_2$ evolved. Gas chromatography can also be used to measure soil respiration by placing soil samples in airtight, sealed containers and periodically sampling the headspace gas to measure $CO_2$ evolved. A gas chromatograph, with a thermal conductivity detector, is used to measure the $CO_2$ concentration after $CO_2$ is separated from other constituents on column materials such as Poropak Q. Computer-operated valves in conjunction with GC allow time-sequence studies to be conducted automatically. Infrared gas analyzers are sensitive to $CO_2$ and can be used for both static and flow systems in the laboratory and in the field after $H_2O$, which adsorbs in the same general wavelength, has been removed. The results are expressed either per unit of soil dry weight ($\mu g\ CO_2$–$C\ g^{-1}$ soil $h^{-1}$) or per unit of microbial biomass (mg $CO_2$–$C\ g^{-1}\ C_{mic}\ h^{-1}$). The ratio of respiration to microbial biomass is termed the metabolic quotient ($qCO_2$) and is in the range of 0.5–3 mg $CO_2$–$C\ g^{-1}\ C_{mic}\ h^{-1}$. The metabolic quotient is particularly useful in differentiating the response of soil biota to sustainable soil management practices. For example, stress, heavy metal pollution, and nutrient deficiency increase $qCO_2$ because microbial biomass decreases and respiration increases.

The measurement of $CO_2$ can also be augmented by incorporating $^{14}C$ or $^{13}C$ into chosen substrates. The tracer may be known molecules such as glucose, cellulose, amino acids, or herbicides or complex materials such as microbial cells or plant residues. The $CO_2$ respired is trapped in alkali as described above. The measurement of $^{14}CO_2$ or $^{13}CO_2$ allows the calculation of the decomposition rate of soil organic matter as well as establishing a balance of the C used in growth relative to substrate decomposition and microbial by-products. A mass spectrometer

capable of directly analyzing a gaseous sample for $^{13}CO_2$ is preferable to the precipitation procedure.

Field studies can be performed by placing an airtight chamber on the soil surface to measure $CO_2$ *in situ*. This procedure does not alter the soil structure and, therefore, field respiration rates of the indigenous microbial population are more reliably measured. Gas samples are taken from the field chamber using a gastight syringe and then injected into a gas chromatograph or infrared analyzer. Measurement of $N_2O$ and $CH_4$ from the same samples is possible. Recently, a new sampling technique was developed to monitor trace gases ($CO_2$, $CH_4$, and $N_2O$) below the soil surface at well-defined depths (Kammann *et al.*, 2001). Probes are constructed from silicone tubing closed with silicone septa on both ends, thereby separating an inner air space from the outer soil atmosphere without direct contact (Fig. 3.2). Gas exchange between the inner and the outer atmosphere takes place by diffusion through the walls of the silicone tube. The advantage of this method is that the silicone probe enables trace gas sampling in wet and waterlogged soils. In general, respiration measurements in the field show much higher analytical variability than those in the laboratory. This is due to higher spatial variability of chemical and physical soil properties and to more variable environmental conditions. Since both soil microorganisms and plant roots contribute to the overall $CO_2$ production in fields, $CO_2$ release in the field has been viewed as a measure of the gross soil metabolic activity.

The net ecosystem exchange (NEE) rate of $CO_2$ represents the balance of gross primary productivity and respiration in an ecosystem. The eddy covariance technique is used to measure the NEE. The covariance between fluctuations in vertical wind velocity and $CO_2$ mixing ratio across the interface between the atmosphere and a plant canopy is measured at a flux tower (Baldocchi, 2003). Although flux tower data represent point measurements with a footprint of typically $1 \times 1$ km they can be used to validate models and to spatialize biospheric fluxes at the regional scale (Papale and Valentini, 2003).

Soil organic matter decomposition in the field can be examined by following the decay process (i.e., weight loss) of added litter. Site-specific litter or standard litter (e.g., wheat straw) is placed in nylon mesh bags, which are placed on or just below the soil surface. Organic matter decomposition can also be followed easily by using a minicontainer-system (Fig. 3.3). The system consists of polyvinylchloride bars as carriers and minicontainers enclosing the straw material, which can be exposed horizontally or vertically in topsoils as well as on the soil surface. The minicontainers are filled with 150 to 300 mg of organic substrate and closed by nylon mesh of variable sizes (20 μm, 250 μm, 500 μm, or 2 mm) to exclude or include the faunal contribution to organic matter decomposition. After an exposure time of several weeks to months, organic matter decomposition is calculated based on the weight loss of the oven-dried material, taking into consideration the ash content of the substrate. An analysis of the time series allows the dynamics of decomposition processes to be investigated.

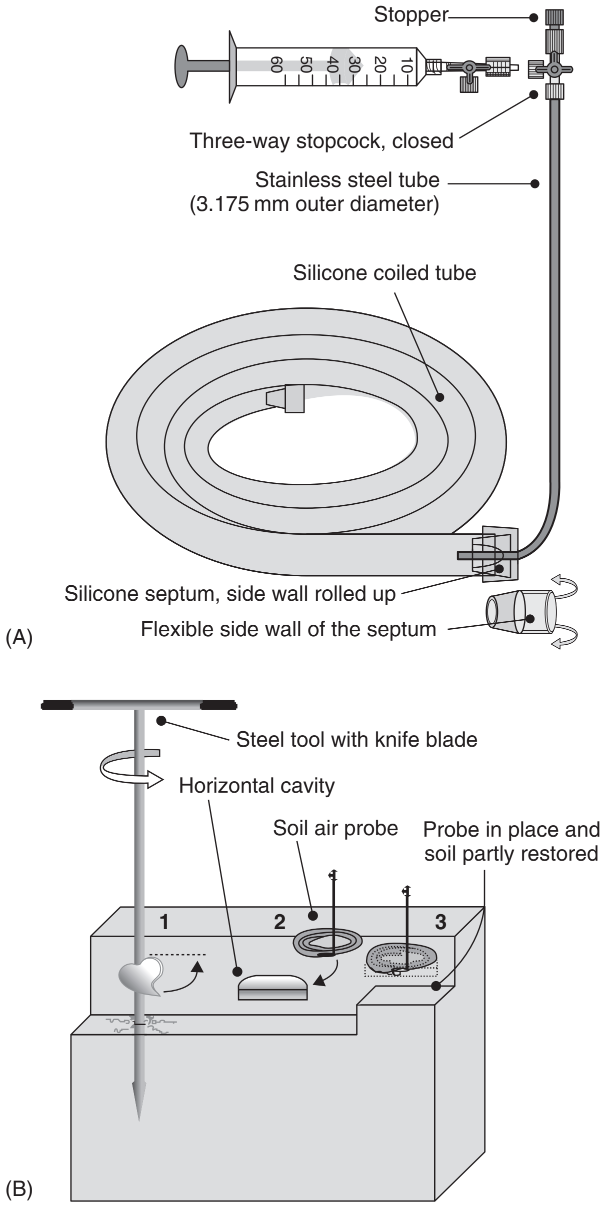



FIGURE 3.2 (A) Sampling technique to monitor concentrations of $CH_4$, $NO_2$, and $CO_2$ in air at well-defined depths using a silicone soil air probe fitted with one stainless steel tube connection. The flat silicone coil is fixed with wire mesh (not shown) to maintain the flat "snail" form. The silicone septum used for the steel tube connection has flexible side walls that can be rolled up and pulled over the silicone tubing to ensure better fixing. (B) Insertion of silicone probe into the soil. After a hole of adequate size is dug (1), the silicone probe is inserted into the hole. After insertion the pit is filled with previously removed soil. Silicone probes can be installed at different soil depths and can also be used in wet or even waterlogged soils (with permission from Kammann *et al.*, 2001).

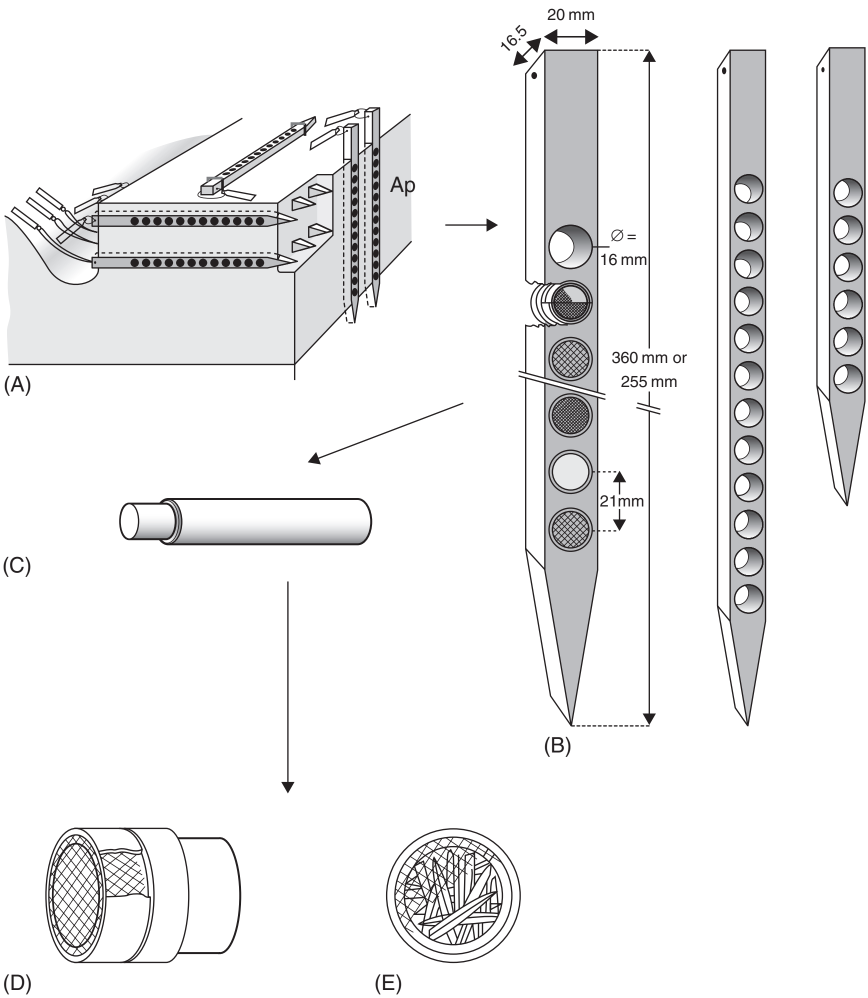



FIGURE 3.3 The decomposition of organic substrates can be estimated by exposing minicontainers filled with site-specific or standard material (e.g., maize straw) for a certain period of time (from weeks to several months) (with permission from Eisenbeis *et al.*, 1999). (A) Minicontainer can be exposed in the top layers of agricultural soils (Ap, ploughed A horizon) and forest soils for several weeks up to years. Vertical insertion of the bars will give information about gradients of decomposition within a soil profile; horizontal exposure of the bars helps to explain spatial variation of decomposition within one horizon. (B) Polyvinylchloride bars are used as carriers for 6 or 12 minicontainers that can be removed from the bar by a rod after exposure (C). (D) A minicontainer in side view, of which the left end is closed with a gauze disc that is fixed with a ring. The mesh size of the gauze will allow or inhibit the colonization of organic substrates by mesofauna (20 µm, 250 µm, 500 µm, or 2 mm). (E) Minicontainers can be filled with organic material of different quality (straw, litter, cellulose, etc.).

## NITROGEN MINERALIZATION

Nitrogen mineralization is estimated in field or laboratory experiments as the release of inorganic N from organic residues of soil organic matter. Alternatively, specific steps of the N mineralization can be estimated (e.g., arginine deaminase, urease, ammonia monooxygenase). Nitrogen availability is measured using aerobic and anaerobic incubation tests as well as soil inorganic N measurements. The recommended methods differ in incubation time and temperature, moisture content, and extraction of ammonium and nitrate. Frequently, soils are incubated under aerobic conditions and analyzed for ammonium, nitrite, and nitrate before and after incubation. Since ammonium is partly immobilized into the microbial biomass during incubation, these incubation methods yield the net production of ammonium and nitrate. Isotope pool dilution techniques enable gross rates of nitrification (or mineralization) to be determined by monitoring the decline in the $^{15}N$ abundance in a nitrate or ammonium pool, labeled at $t = 0$, and receiving unlabeled nitrogen via nitrification or mineralization, respectively (Murphy *et al.,* 2003). Labeled N can be applied as $^{15}NH_4^+$ solution or injected as $^{15}NH_3$ gas into soil. The use of $^{15}N$ pool dilution and enrichment can also be used to separate the heterotrophic and autotrophic pathways of nitrification. An isotopic dilution experiment using $^{14}NH_4{}^{15}NO_3$ yielded rates of nitrification by the combined autotrophic and heterotrophic paths. A parallel isotope dilution experiment with $^{15}NH_4{}^{15}NO_3$ provided the gross mineralization rate and the size and $^{15}N$ abundance of the nitrate pool at different time intervals. Spatial variability of the tracer addition and extraction must be taken into account in interpreting such data.

## ACTIVITIES AND LOCATIONS OF ENZYMES

Enzymes are specialized proteins that combine with a specific substrate and act to catalyze a biochemical reaction. In soils, enzyme activities are essential for energy transformation and nutrient cycling. The enzymes commonly extracted from soil, and their range of activities, are given in Table 3.7. Some enzymes (e.g., urease) are constitutive and routinely produced by cells; others such as cellulase are adaptive or induced, being formed only in the presence of a compatible substrate or some other initiator or in the absence of an inhibitor. Dehydrogenases are often measured because they are found only in living systems. Enzymes associated with proliferating cells occur in the cytoplasm, the periplasm, and the cell membrane. Figure 3.4 shows that soil enzymes are not only associated with proliferating cells but also associated, as extracellular enzymes, with humic colloids and clay minerals.

Standardized methods for a broad range of enzymes are described by Tabatabai (1994), Alef and Nannipieri (1995), and Schinner *et al.* (1996). A general introduction to enzymes in the environment and their activity, ecology, and applications is given by Burns and Dick (2002). This section will focus primarily on

TABLE 3.7 Some Enzymes Extracted from Soils, the Reactions They Catalyze, and Their Ranges of Activity[a]

| Enzyme | Reaction | Range of activity |
|---|---|---|
| Cellulase | Endohydrolysis of 1,4-β-glucosidic linkages in cellulose, lichenin, and cereal β-glucose | 0.4–80.0 μM glucose $g^{-1}$ 24 $h^{-1}$ |
| β-Fructofuranosidase (invertase) | Hydrolysis of terminal nonreducing β-D-fructofuranoside residues in β-fructofuranosides | 0.61–130 μM glucose $g^{-1}$ $h^{-1}$ |
| β-Glucosidase | Hydrolysis of terminal, nonreducing β-D-glucose residues with release of β-D-glucose | 0.09–405 μM *p*-nitrophenol $g^{-1}$ $h^{-1}$ |
| Proteinase | Hydrolysis of proteins to peptides and amino acids | 0.5–2.7 μM tyrosine $g^{-1}$ $h^{-1}$ |
| Urease | Hydrolysis of urea to $CO_2$ and $NH_4^+$ | 0.14–14.3 μM N–$NH_3$ $g^{-1}h^{-1}$ |
| Alkaline phosphatase | Orthophosphoric monoester + $H_2O$ → an alcohol + orthophosphate | 6.76–27.3 μM *p*-nitrophenol $g^{-1}$ $h^{-1}$ |
| Acid phosphatase | Orthophosphoric monoester + $H_2O$ → an alcohol + orthophosphate | 0.05–86.3 μM *p*-nitrophenol $g^{-1}$ $h^{-1}$ |
| Arylsulfatase | A phenol sulfate + $H_2O$ → a phenol + sulfate | 0.01–42.5 μM *p*-nitrophenol $g^{-1}$ $h^{-1}$ |
| Catalase | $2\ H_2O_2 \rightarrow O_2 + 2H_2O$ | 61.2–73.9 μM $O_2$ $g^{-1}$ 24 $h^{-1}$ |

[a]Adapted from Tabatabai and Fung (1992) and Nannipieri *et al.* (2002).

colorimetric and fluorimetric techniques to measure enzyme activities in soils and on some approaches to visualize the location of soil enzymes. Since many of the enzymes that are frequently measured can be intracellular, extracellular, bound, and/or stabilized within their microhabitat, assay results must be interpreted with caution. Therefore, most assays determine enzymatic potential and not necessarily the activity of proliferating microorganisms.

## SPECTROPHOTOMETRIC METHODS

Many substrates and products of enzymatic reactions absorb light either in the visible or in the ultraviolet region of the spectrum or can be measured by a simple color reaction. Due to their higher sensitivity, methods based on the analysis of the released product are more frequently used than methods based on the analysis of substrate depletion. Analyzing enzyme activities involves incubating soils with

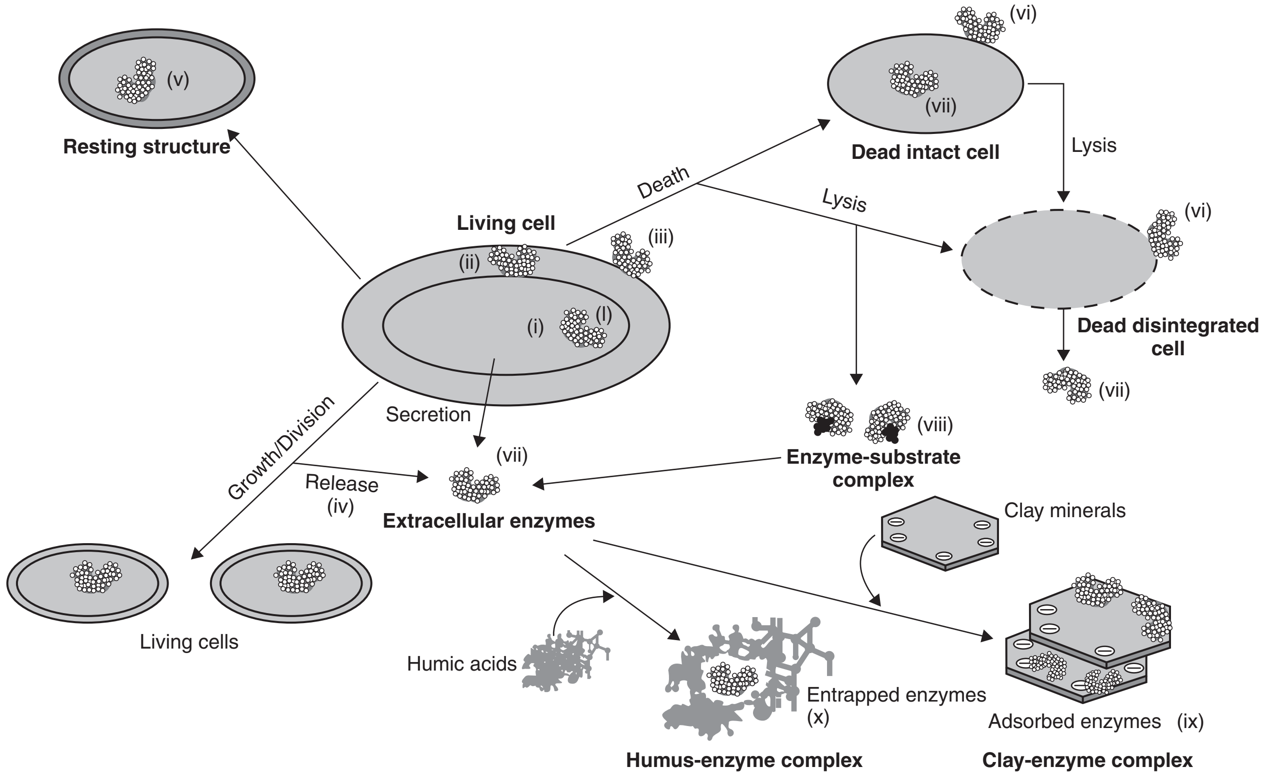



FIGURE 3.4 Locations of enzymes (from Burns, 1982, adapted by Klose, 2003). (i) Intracellular enzymes. (ii) Periplasmatic enzymes. (iii) Enzymes attached to outer surface of cell membranes. (iv) Enzymes released during cell growth and division. (v) Enzymes within nonproliferating cells (spores, cysts, seeds, endospores). (vi) Enzymes attached to dead cells and cell debris. (vii) Enzymes leaking from intact cells or released from lysed cells. (viii) Enzymes temporarily associated in enzyme– substrate complexes. (ix) Enzymes absorbed to surfaces of clay minerals. (x) Enzymes complexed with humic colloids. (Reproduced with permission from Taylor & Francis Group, LLC.)

the respective substrate at specific temperature, pH, and time; subsequently extracting the product; and then colorimetrically determining its concentration.

Dehydrogenases, which are intracellular enzymes catalyzing oxidation–reduction reactions, can be detected using a water-soluble, almost colorless tetrazolium salt, which forms a reddish formazan product after an incubation period of several hours. A commonly used tetrazolium salt is 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium chloride (INT), which is transformed into an intensely colored, water-insoluble formazan (INT-formazan). The production of INT-formazan can be detected spectrophotometrically by quantifying total INT-formazan production. Another enzyme assay system used to reflect general or total microbial activity in soil samples is based on the hydrolysis of fluorescein diacetate. Fluorescein diacetate is hydrolyzed by a wide variety of enzymes including esterases, proteases, and lipases.

Enzymes involved in C cycling (xylanase, cellulase, invertase, and trehalase) are measured based on the release of sugars after incubating soils with a buffered solution (pH 5.5) containing their corresponding substrates (xylan, carboxymethylcellulose, sucrose, or trehalose). The incubation period depends on the substrate used: high-molecular-weight substrates are incubated for 24 h, whereas low-molecular-weight substrates are incubated for only 1 to 3 h. Reducing sugars released during the incubation period cause the reduction of potassium hexacyanoferrate(III) in an alkaline solution. Reduced potassium hexacyanoferrate(II) reacts with ferric ammonium sulfate in an acid solution to form a complex of ferric hexacyanoferrate(II) (Prussian blue), which is determined colorimetrically.

Enzymes involved in P cycling (phosphomonoesterase, phosphodiesterase, phosphotriesterase) are preferably determined after the addition of a substrate analog, *p*-nitrophenyl phosphate. Phosphomonoesterase hydrolyzes the phosphate–ester bond of the *p*-nitrophenyl phosphate, and the *p*-nitrophenol released is yellow under alkaline conditions. The concentration can be determined spectrophotometrically against a standard curve. For determining arylsulfatase, which catalyzes the hydrolysis of organic sulfate esters, *p*-nitrophenyl sulfate is used as a substrate analog. The widespread use of *p*-nitrophenyl substrates for different enzyme assays is due to the possibility of quantitatively extracting the released product *p*-nitrophenol. Urease is an important extracellular enzyme that hydrolyzes urea into $CO_2$ and $NH_3$. The urease assay involves measuring the released ammonia either by a colorimetric procedure or by steam distillation followed by a titration assay of ammonia.

## FLUORESCENCE METHODS

Fluorogenic substrates are used to assay extracellular enzymes in aquatic and terrestrial environments. These substrates contain an artificial fluorescent molecule and one or more natural molecules (e.g., glucose, amino acids) and they are linked by a specific binding (e.g., peptide binding, ester binding). The substrates used are conjugates of the highly fluorescent compounds 4-methylumbelliferone

(MUB) and 7-amino-4-methyl coumarin (AMC). Marx *et al.* (2001) used this method to measure the activities of enzymes involved in C cycling (β-D-glucosidase, β-D-galactosidase, β-cellobiase, β-xylosidase), N cycling (leucine aminopeptidase, alanine aminopeptidase, lysine–alanine aminopeptidase), P cycling (acid phosphatase), and S cycling (arylsulfatase). Fluorogenic model substrates are not toxic and they are supplied to soil suspensions in high or increasing quantities to measure the maximum velocity of hydrolysis ($V_{max}$). Fluorescence is observed after enzymatic splitting of the complex molecules (Fig. 3.5). The increasing interest of soil microbiologists in the use of fluorogenic substrates to measure soil enzyme activities is mainly because of their high sensitivity. A comparative study between a fluorimetric and a standard colorimetric enzyme assay based on *p*-nitrophenyl substrates generated similar values for the maximum rate of phosphatase and β-glucosidase ($V_{max}$), but the affinity for their respective substrates (as indicated by $K_m$ values, Michaelis–Menten constant) was up to two orders of magnitude greater for the 4-methylumbelliferyl substrates compared to the *p*-nitrophenyl substrates (Marx *et al.*, 2001). This high sensitivity of fluorimetric enzyme assays provides an

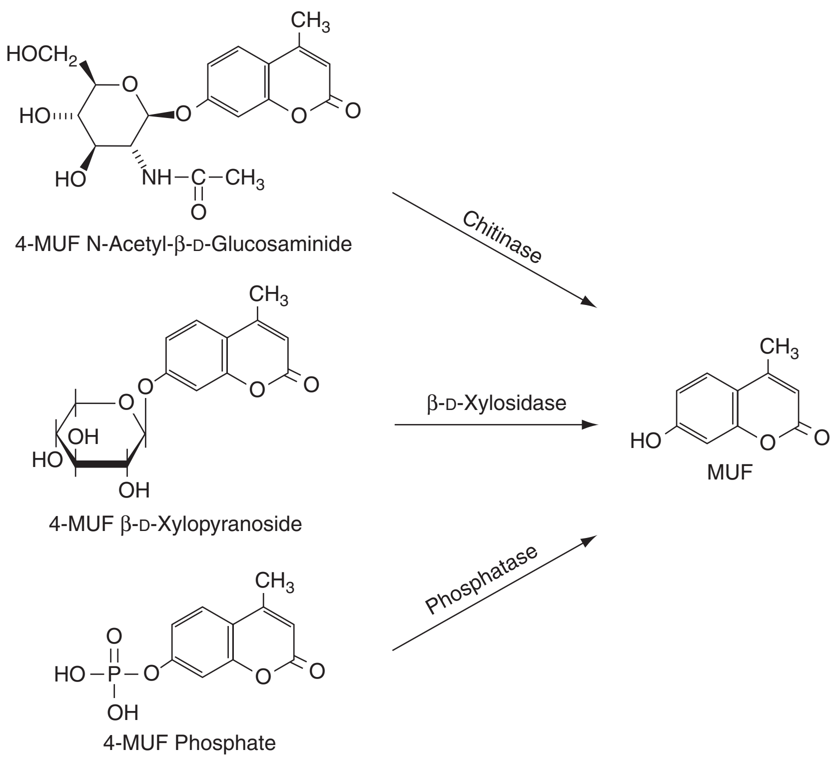



FIGURE 3.5 Enzyme assays using different nonfluorescent substrates and measuring highly fluorescent products after a short-term incubation.

opportunity to detect enzyme activities of small sample sizes (e.g., microaggregates and rhizosphere samples) and/or low activity (e.g., samples of subsoil, peat, and soil solutions). The development of an improved automated assay using a 96-well microplate reader provides a convenient system for processing large numbers of different samples; it also allows the activity rates to be measured for a range of enzymes in a number of soils with an appropriate number of replicates (Vepsäläinen *et al.*, 2001). Alternatively, a multiple-substrate approach is available, which is based on the simultaneous measurement of seven enzyme activities estimated after incubation, separation of the nonhydrolyzed 4-MUF and 7-AMC substrates by HPLC, and quantification by UV-absorption at 320 nm (Stemmer, 2004).

### TECHNIQUES FOR IMAGING THE LOCATION OF ENZYMES

Thin-section techniques are combined with histochemical and imaging techniques to visualize the location of enzymes and their activities. Early papers by Foster *et al.* (1983) showed peroxidase, succinic dehydrogenase, and acid phosphatase bound to roots, bacterial cell walls, and organic matter in soil by using either transmission electron microscopy or scanning electron microscopy. In the future, atomic force microscopy, which measures small-scale surface topographical features, will help us to understand enzyme–clay interactions. Confocal microscopy has the potential to reconstruct the detailed three-dimensional distribution of enzyme proteins in soils.

Enzymatic properties of single cells have been screened in the aquatic environment, biofilms, and activated sludge by a new type of fluorogenic compound, ELF-97 (Molecular Probes, Eugene, OR, USA), which is combined with sugar, amino acid, fatty acid, or inorganic compounds such as sulfate or phosphate. This substrate is converted to a water-insoluble, crystalline, fluorescent product at the site of enzymatic hydrolysis, thus reporting the location of active enzymes when viewed by fluorescence microscopy. Kloeke and Geesey (1999) combined this technique with a 16S rRNA oligonucleotide probe specific for the *Cytophaga–Flavobacteria* group to prove the importance of these microorganisms in liberating inorganic orthophosphate in discrete, bacteria-containing areas of the floc matrix in aerobic activated sludge (Fig. 3.6). Phosphatase activity was primarily localized in the immediate vicinity of the bacterial cells rather than dispersed throughout the floc or associated with rotifers (see Figs. 3.6A and 3.6B). These new substrates have the potential to be used for visualizing the location of enzyme activities in microenvironments in soil.

## FUNCTIONAL DIVERSITY

Microbial diversity reflects the variation in species assemblages within a community. A broader view of functional diversity has advanced our understanding of

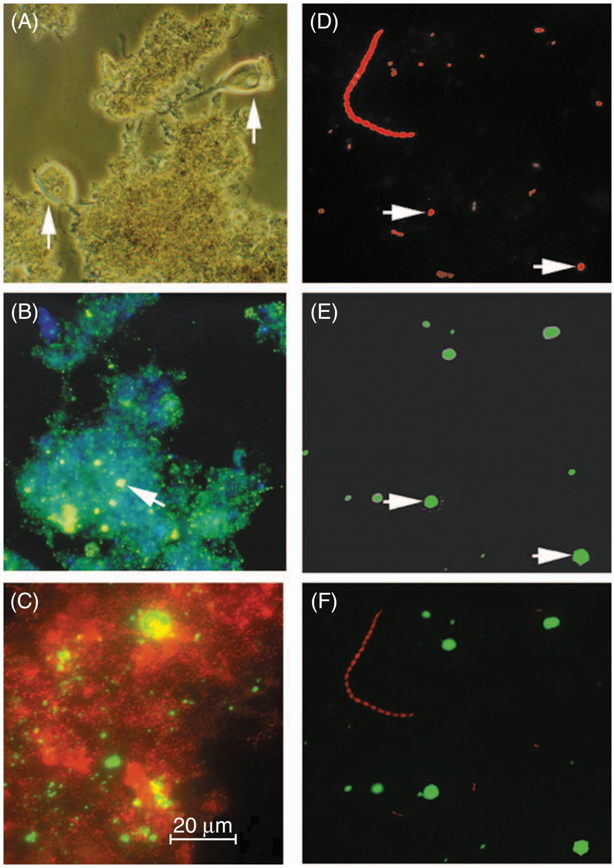



FIGURE 3.6 (A) Phase-contrast photomicrograph of activated sludge floc material and associated microorganisms. Arrows indicate protozoa grazing on the floc particles. (B) Epifluorescence photomicrograph of the same field of view as in (A) of whole floc material revealing the areas of intense phosphatase activity (yellow spots where ELF crystals have precipitated, arrow). (C) Epifluorescence photomicrograph of thin section of a floc particle stained red-orange with acridine orange, revealing discrete regions within the floc displaying phosphatase activity (green spots) as a result of incubation in the presence of ELP-P. (D) Epifluorescence photomicrograph image of a homogenized activated sludge filtrate showing floc bacteria from the *Cytophaga–Flavobacteria* group by using a filter combination that reveals cells that react with CF319a oligonucleotide probe (red). (E) Epifluorescence photomicrograph of the same field of view as in (D) using a filter combination that resolves crystals of ELF (green spots), indicating areas of phosphatase activity. (F) Merged image of (D) and (E), revealing the subpopulation of cells that react positively with the CF319a and also display phosphatase activity (according to Kloeke and Geesey, 1999; pictures are reproduced with the permission of Springer-Verlag).

the significance of biodiversity to biochemical cycling. This includes several levels of resolution: (1) the importance of biodiversity to specific biogenic transformations, (2) the complexity and specificity of biotic interactions in soils that regulate biogeochemical cycling, and (3) how biodiversity may operate at different hierarchically

arranged spatial and temporal scales to influence ecosystem structure and function (Beare *et al.*, 1995). Most methods used for measuring functional diversity consider only the importance of biodiversity of those groups that regulate biochemical cycling. Several approaches enable functional diversity to be measured in situations in which taxonomic information is poor. These include using binary biochemical and physiological descriptors to characterize isolates, evaluating enzymatic capabilities for metabolizing particular substrates, extracting DNA and RNA from the soil, and probing genes that code for functional enzymes. Recent advances in genomic analysis and stable isotope probing are the first steps toward resolving a better linkage between structure and function in microbial communities (see also Chaps. 4, 7, and 8).

Commercially available Biolog bacterial identification system plates or community-level physiological profiles (CLPP) have been used to assess functional diversity of microorganisms, based on utilization patterns of a wide range (up to 128) of individual C sources. The culturable subpart of the microbial community, which exhibits the fast growth rates typical of r-strategists, primarily contributes to CLPP analysis. Preston-Mafham *et al.* (2002) assessed the pros and cons of its use and point out inherent biases and limitations and possible ways of overcoming certain difficulties. A modification of the SIR method (Degens and Harris, 1997) involves determining patterns of *in situ* catabolic potential as a measure of functional diversity. These profiles are determined by adding a range of simple C substrates to the soils and measuring short-term respiration response. The use of whole soil in a microplate assay as described by Campbell *et al.* (2003) can also be used to explore the metabolic capacity of the soil microbial community. An alternative approach was proposed by Kandeler *et al.* (1996), using the prognostic potential of 16 soil microbial properties, including microbial biomass, soil respiration, N-mineralization, and analyses for 13 soil enzymes involved in cycling of C, N, P, and S. Multivariate statistical analysis is used to calculate the functional diversity from measured soil microbial properties. The latter approach is based on the following assumptions: The composition of the microbial species assemblage (taxonomic diversity) determines the community's potential for enzyme synthesis. The actual rate of enzyme production and the fate of produced enzymes are modified by environmental effects as well as by ecological interactions. The spectrum and amount of active enzymes are responsible for the functional capability of the microbial community irrespective of being active inside or outside the cell. Presence or absence of a certain function, as well as the quantification of the potential of the community to realize this function, has to be considered in ecological studies. This approach may permit evaluation of the status of changed ecosystems (e.g., by soil pollution, soil management, global change) while providing insight into the functional diversity of the soil microbial community of the undisturbed habitat.

Physiological methods are applied to understand the physiology of single cells and soil biological communities, as well as biogeochemical cycling in terrestrial ecosystems. Small-scale studies explain biological reactions in aggregates, in the

rhizosphere, or at the soil–litter interface. Combining physiological and molecular methods helps us to understand gene expression, protein synthesis, and enzyme activities at the micro- and nanoscales. Linking these methods can also explain whether the abundance and/or the function of organisms is affected by soil management, environmental change, or soil pollution.

At the field scale, researchers use biochemical and physiological methods to investigate the functional response of soil organisms to the manipulation or preservation of soils. These applications include microbe–plant interactions and controlling plant pathogens, as well as understanding organic matter decomposition and its impact on local and global C and N cycling. Soil biologists investigate the effects of soil management (tillage, fertilizer, pesticides, crop rotation) or disturbance on the function of soil organisms. In many cases, soil microbial biomass and/or soil microbial processes can be early predictors of the effects of soil management on soil quality and can indicate the expected rapidity of these changes. Monitoring of soil microbial properties is also included in environmental studies that test the use of soil microorganisms in bioremediation and composting. Future challenges in functional soil microbiology are to use our present knowledge to scale-up these data to the regional and global scale.

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# 4

# Molecular Methods for Studying Soil Ecology

Janice E. Thies



## INTRODUCTION

No other area of soil ecology has developed more rapidly in recent years than the use of molecular methods to characterize the soil microbial community. The ability to extract deoxyribonucleic acids (DNA) and ribonucleic acids (RNA) from cells contained within soil samples and their direct analysis in hybridization experiments or use in polymerase chain reaction (PCR) amplification experiments have allowed us to detect and begin to characterize a vast diversity of microbes unimagined previously. Direct microscopic counts of soil bacteria are typically one to two orders of magnitude higher than counts obtained by culturing (Chap. 5). Molecular

methods have the potential to provide access to this, as yet undescribed, 90–99% of the soil community.

Molecular microbial ecology relies on extracting and characterizing nucleic acids and other subcellular components, such as phospholipid fatty acids (Chap. 3), from soil organisms. Once extracted, nucleic acids or other marker molecules may be analyzed directly; or for DNA, specific target sequences may be amplified by PCR and the resulting PCR products characterized further. In the case of RNA (ribosomal (rRNA) or messenger (mRNA)), complementary DNA (cDNA) is derived from the RNA extract by reverse transcriptase (RT) PCR and the cDNA produced is analyzed subsequently. Both extracting nucleic acids from soil and amplifying them by PCR may have considerable biases associated with them and these must be taken into account when interpreting the results of subsequent analyses.

The aim of many molecular community analyses is to describe population diversity by calculating taxon richness and evenness. Due to bias in DNA and RNA extraction and PCR amplification, it is difficult, if not impossible, to assess the true abundance of different taxa using these approaches. In addition, these methods alone, although very powerful, cannot be used to assign function unambiguously to different taxa. Hence, molecular methods should be used in concert with other approaches (termed a polyphasic or multiphasic approach) to achieve a more holistic understanding of the structure and function of soil microbial communities. The focus of this chapter is on methods for extracting and analyzing soil- and sediment-derived nucleic acids and drawing ecological information from analysis results.

## TYPES AND STRUCTURES OF NUCLEIC ACIDS

There are two types of nucleic acids present in all cells: DNA and RNA. These are the target molecules for most molecular analyses. The structure of DNA was deduced by Watson and Crick in 1953. They described a double helix of nucleotide bases that could "unzip" to make copies of itself. DNA was known to contain equimolar ratios of adenine (A) and thymine (T) and of cytosine (C) and guanine (G). Watson recognized that the adenine–thymine pair, held together by two hydrogen bonds, and the cytosine–guanine pair, held together by three hydrogen bonds, resulted in similar shapes and could fit together to form the rungs of a ladder of nucleotides. Molecular methods employing nucleic acids take advantage of the base-pairing rules between these four nucleotides (Fig. 4.1).

The DNA backbone is composed of deoxyribose (sugar), phosphates, and the associated purine (A and G) and pyrimidine (T and C) bases. It is the base-pairing specificity between the nucleotides that leads directly to the faithful copying of both strands of the DNA double helix during replication and that can be exploited to make copies of selected genes (or regions of the DNA molecule) *in vitro* by use of the PCR.

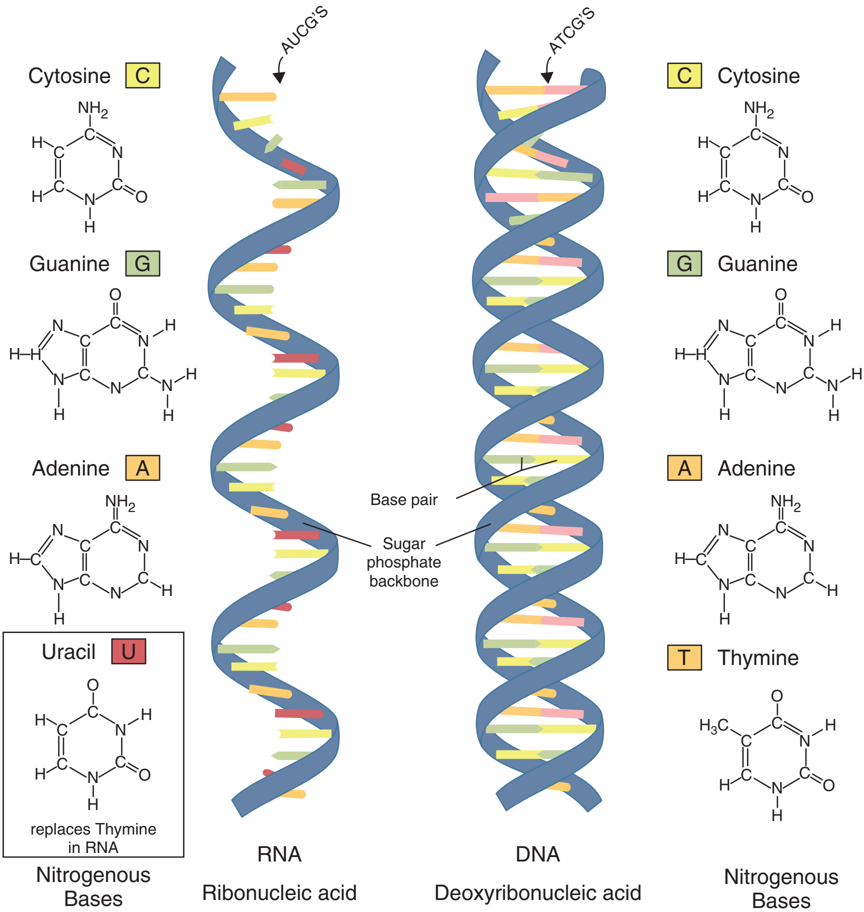



FIGURE 4.1 The basic structure of DNA and RNA. DNA is double-stranded, whereas RNA is single-stranded. The nucleotide, thymine, in DNA is replaced with uracil in RNA. Image adapted from the National Human Genome Research Institute Talking Glossary of Genetic Terms, available at http://www.genome.gov/Pages/Hyperion/DIR/VIP/Glossary/Illustration/rna.cfm?key=ribonucleic%20acid%20%28RNA%29.

Base-pairing specificity in DNA also allows for the transcription of genes coding for rRNA to produce ribosomes, which facilitate protein synthesis; mRNA, which carries the genetic instructions for protein assembly; and tRNA, which is required for transporting and linking amino acids during protein assembly (Fig. 4.2) (Campbell and Reece, 2005). RNA transcripts are single-stranded, contain ribose instead of deoxyribose, and substitute uracil (U) for thymine in the nucleic acid sequence, thus necessitating reverse transcription to cDNA to analyze RNA extracted from soils. Most molecular analyses target either DNA or rRNA, although limited analysis of mRNA from environmental samples has become possible.

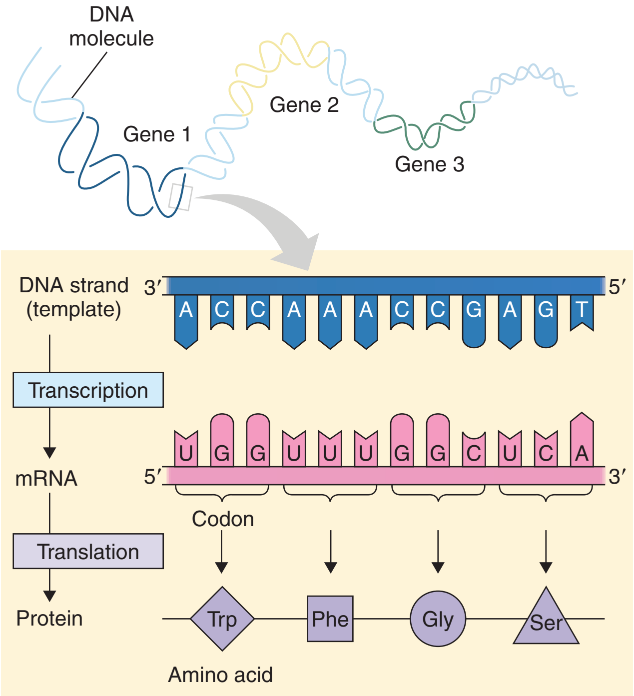



FIGURE 4.2 The relationship between DNA gene sequence, transcription into mRNA, and translation into the coded protein sequence (with permission from Addison Wesley Longman, Inc., 1999).

## USE OF NUCLEIC ACID ANALYSES FOR SOIL ECOLOGY STUDIES

A wide range of techniques are available for nucleic acid analysis. These techniques fall into three basic categories: (i) analysis of nucleic acids *in situ*, (ii) direct analysis of extracted DNA/RNA, and (iii) analysis of PCR-amplified segments of the DNA molecule. In PCR-based approaches, the primers chosen dictate the target for amplification, such as rRNA genes or genes that code for proteins with functions of ecological interest (those involved in nitrogen fixation, ammonia or methane oxidation, or denitrification). Some of the techniques used more commonly and their interrelationships with each other and traditional methods of microbial analysis are shown in Fig. 4.3. Most methods require that target molecules be separated from the soil matrix prior to analysis. A few techniques, such as fluorescence *in situ* hybridization (FISH), do not. Much of our ability to make sense of results of molecular analyses and to design more robust methods depends on the continuing development of nucleic acid and protein sequence databases and associated bioinformatics analytical tools. Through comparative sequence analysis, targeted primers for use in PCR assays and probes for use in hybridization studies can be designed that have the required level of specificity for a given study.

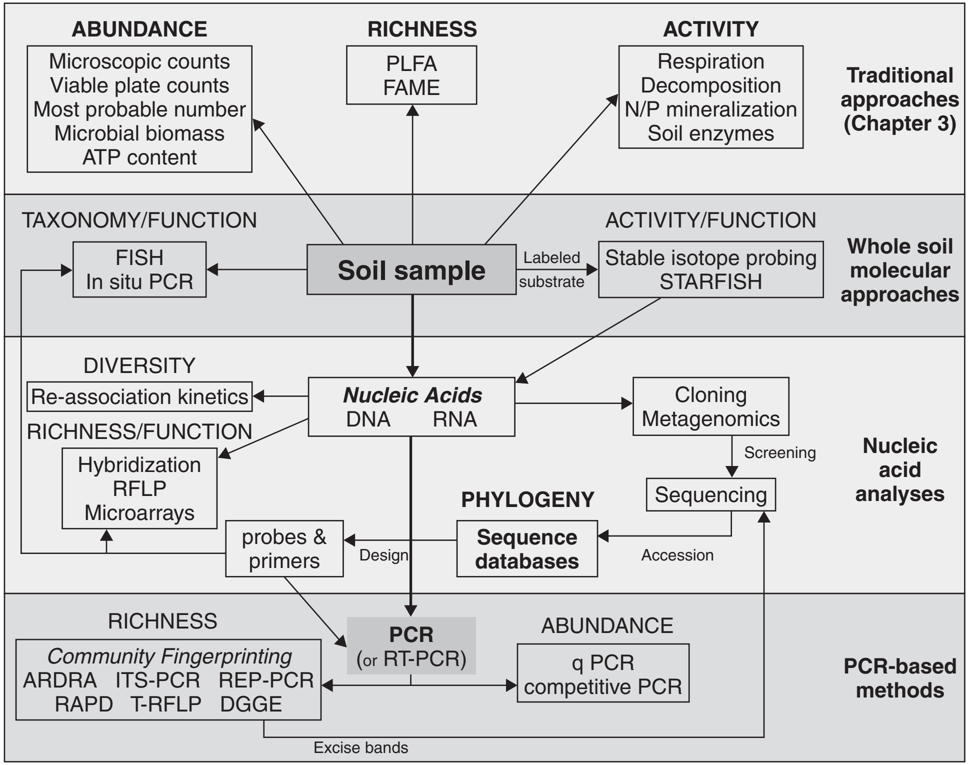



FIGURE 4.3 Overview of traditional and molecular techniques used in soil ecology studies.

## DIRECT MOLECULAR ANALYSIS OF SOIL BIOTA

### NUCLEIC ACID HYBRIDIZATION

Nucleic acid hybridization involves bonding together short complementary nucleic acid strands (probes) to a target sequence. The probe is generally labeled with a radioisotope or fluorescent molecule, and the target sequence is typically bound to a nylon membrane or other solid surface. A positive hybridization signal is obtained when complementary base pairing occurs between the probe and the target sequence. After any unbound probe is removed, a positive signal is visualized by exposing the hybridized sample to X-ray film, in the case of radiolabeled probes, or by use of fluorescence microscopy in the case of fluorescent probes. The type of probe used and the way the probe is labeled determine the applications of nucleic acid hybridization techniques. Table 4.1 provides examples of probes that are used commonly to address specific questions about the presence and location of selected organisms in a soil community. Further details on this approach can be found in Amann *et al.* (1995). The GenBank (http://www.ncbi.nlm.nih.gov/) sequence database and the Ribosomal Database Project II (http://rdp.cme.msu.edu/html/) have tools to assist users to design hybridization probes for use in molecular soil ecology studies. Information about probes that have already been designed for specific purposes can be found at the ProbeBase Web site (http://www.microbial-ecology.net/probebase/).

Techniques based upon nucleic acid colony hybridization (colony blotting) have particular value in rapidly screening bacterial isolates for their identity, such as identifying specific rhizobia strains occupying root nodules or screening libraries containing DNA clones obtained from a soil community. Nucleic acid hybridization probes can also be used to detect specific phylogenetic groups of bacteria in appropriately prepared soil samples. In the latter application, a nucleic acid probe is fluorescently labeled and hybridized to target sequences contained within microbial cells *in situ* using the FISH technique. These protocols have been described extensively in reviews by Amann *et al.* (1995) and Amann and Ludwig (2000).

TABLE 4.1 Hybridization Probes Used in the Analysis of Soil Microbial Communities

| Probe name | Target gene | Community target | Reference |
|---|---|---|---|
| EUB338 | 16S rRNA | Most Bacteria | Amann *et al.*, 1990 |
| UNIV 1389b | 18S rRNA | Most Eucarya | Zheng *et al.*, 1996 |
| UNIV 1389c | 16S rRNA | Archaea | Zheng *et al.*, 1996 |
| BET42a | 23S rRNA | $\beta$-Proteobacteria | Manz *et al.*, 1992 |
| Beta-AO233 | 16S rRNA | Ammonium oxidizers | Stephen *et al.*, 1998 |

Some probes are “universal” in that they target all known organisms in the Domain Bacteria or the Domain Archaea. Other probes are used to target different divisions within the Domain Bacteria or genes with specific functions that are unique to a specific class of bacteria.

The FISH technique employs an oligonucleotide probe conjugated with a fluorescent molecule (or fluorochrome). The probe is designed to bind to complementary sequences in the rRNA of the 16S subunit of the ribosomes within bacterial cells. Because metabolically active cells contain a large number of ribosomes, the concentration of fluorescently labeled probe is relatively high inside the cells, causing them to fluoresce under UV light. The final result is high binding specificity and typically low background fluorescence. For simultaneous counting of subpopulations in a given sample, probes can be designed that bind to specific sequences of rRNA that are found only in a particular group of organisms (i.e., Archaea, Bacteria, or subdivisions of the Bacteria; see Table 4.1) and used in conjunction with one another. FISH can also be combined with microautoradiography to determine specific substrate uptake profiles for individual cells within complex microbial communities (STARFISH, substrate-tracking autoradiographic fluorescence *in situ* hybridization; Ouverney and Fuhrman, 1999; Lee *et al.*, 1999). Because these methods label only metabolically active cells, the samples can be labeled simultaneously with dyes that bind to nucleic acids, such as 4′,6-diamidino-2-phenylindole (DAPI) or acridine orange, to facilitate a total cell count using fluorescence microscopy (Li *et al.*, 2004). FISH is useful especially when used in conjunction with confocal laser scanning microscopy (CLSM; see below) as it allows three-dimensional visualization of the relative positions of diverse populations, even within complex communities such as biofilms and the surfaces of soil aggregates (Binnerup *et al.*, 2001). The key advantage of FISH is the ability to visualize and identify organisms on a microscale in their natural environment. Such techniques have enormous potential for studying microbial interactions with plants and the ecology of target microbial populations in soil; however, the binding of fluorescent dyes to organic matter resulting in nonspecific fluorescence is a common problem in soils with high organic matter contents, such as peats, or other particles with high surface charge, such as black carbon. Image analysis software is readily available and may be "trained" to detect only those aspects of an image that meet specified criteria.

## CONFOCAL MICROSCOPY

CLSM, combined with *in situ* hybridization techniques, has been applied with considerable success to visualize the structure of soil microbial communities. The basic principle of CLSM is to first create an image that is composed only of emitted fluorescence signals from a single plane of focus. This is done using a pinhole aperture, which eliminates any signal that may be coming from portions of the field that are out of focus. A series of these optical sections is scanned at specific depths and then each section is "stacked" using imaging software, giving rise to either a two-dimensional image that includes all planes of focus in the specimen or a computer-generated three-dimensional image. This gives us unprecedented resolution in viewing environmental specimens, allowing for better differentiation of organisms from particulate matter as well as giving us an insight into the three-dimensional spatial relationships of microbial communities within their environment (Fig. 4.4).

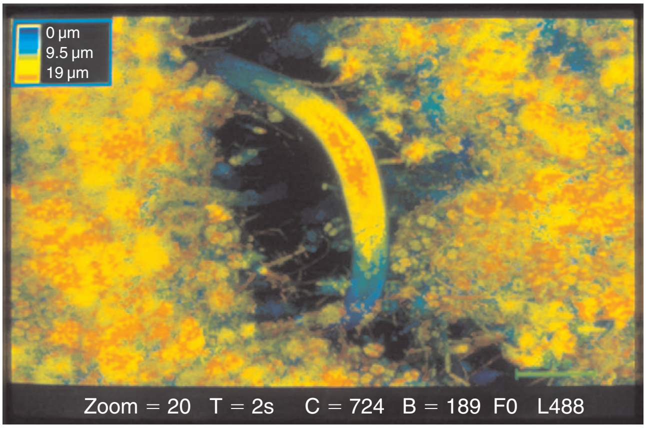



FIGURE 4.4 Confocal laser scanning microscopy image of a nematode feeding on a bacterial biofilm (with permission from W. C. Ghiorse, Cornell University).

## BIOSENSORS AND MARKER GENE TECHNOLOGIES

Introduced marker genes, such as *lux*AB (luminescence), *lac*Z ($\beta$-galactosidase), and *xyl*E (catechol 2, 3-dioxygenase) are now being used more frequently in soil microbial ecology studies (Table 4.2). One such gene that has attracted a lot of attention in rhizosphere studies is *gfp,* which encodes the green fluorescent protein (GFP). Green fluorescent protein is a unique bioluminescent genetic marker, which can be used to identify, track, and count specific organisms into which the gene has been cloned that have been reintroduced into the environment (Chalfie *et al.*, 1994). The *gfp* gene was discovered in and is derived from the bioluminescent jellyfish *Aequorea victoria* (Prasher *et al.*, 1992). Once cloned into the organism of interest, GFP methods require no exogenous substrates, complex media, or expensive equipment to monitor and, hence, are favored over many fluorescence methods for environmental applications (Errampalli *et al.*, 1999). GFP-marked cells can be identified using a standard fluorescence microscope fitted with excitation and emission filters of the appropriate wavelengths. One reason for such keen interest in GFP is that there is no background GFP activity in plants or the bacteria and fungi that interact with them, thereby making *gfp* an excellent target gene that can be introduced into selected bacterial or fungal strains and used to study plant–microbe interactions (Errampalli *et al.*, 1999). Basically, *gfp* is transformed into either the chromosome or a plasmid in a bacterial strain, where it is subsequently replicated. Various gene constructs have been made, which differ in the type of promoters or terminators used, and some contain repressor genes such as *lac*I for control of *gfp* expression. Once key populations in a sample are known and isolates obtained, they can be subsequently marked with *gfp* or other genes producing detectable products in order to track them and assess their functions and interactions in soil and the rhizosphere. In addition to GFP, red-shifted and yellow-shifted variants have been

TABLE 4.2 Major marker methods used for bacterial detection (modified from Errampalli *et al*., 1999).

| Marker | Substrate required | Detection principle | Quantification method(s) | Other features |
|---|---|---|---|---|
| *gfp* | no | fluorescence | epifluorescence microscopy; fluorimetry; flow cytometry; plate count | single-cell detection; real-time *in situ* detection |
| *lux*AB/*luc* | *n*-decanal/ luciferin | luminescence | luminometry; CCD digitized camera; flow cytometry; plate count | single-cell detection; real-time *in situ* detection |
| *lac* ZY | x-gal | colony color change | plate count (colorless → blue) | |
| *xyl* B | catechol | colony color change | plate count (colorless → yellow) | |
| heavy metal resistance genes | heavy metals | heavy metal resistance | plate count | |
| antibiotic resistance genes | antibiotics | antibiotic resistance | plate count | |

described. Development of *gfp* mutants with a series of different excitation and emission wavelengths makes it possible to identify multiple bacterial populations simultaneously. The *gfp* gene has been introduced into *Sinorhizobium meliloti, Pseudomonas putida*, and *Pseudomonas* sp., among other common soil bacteria, and used in soil ecology studies. Marked strains can be visualized in infection threads, root nodules, and colonized roots and even inside digestive vacuoles of protozoa (Fig. 4.5). If the *gfp* gene is cloned along with specific promoters, such as the *mel*A ($\alpha$-galactosidase) promoter, then they can be used as biosensors to report back to the observer if the inducers, in this case galactosides, are present and at what relative concentration in the surrounding environment (Bringhurst *et al.,* 2002).

Marker gene approaches are restricted to use in organisms that can be manipulated in culture. While considerable information can be gained about how marked microbes interact with soil colloids and other soil organisms, and can be used as biosensors for detecting environmental concentrations of various compounds, they do not yield information about the vast, unknown majority of soil microbes for which cultured representatives have yet to be obtained.

## EXTRACTION OF NUCLEIC ACIDS (DNA/RNA)

Both whole-community nucleic acid analyses and those based on PCR amplification of target genes require that the nucleic acids contained in a soil sample be separated from the solid phase. A variety of methods used to extract nucleic acids

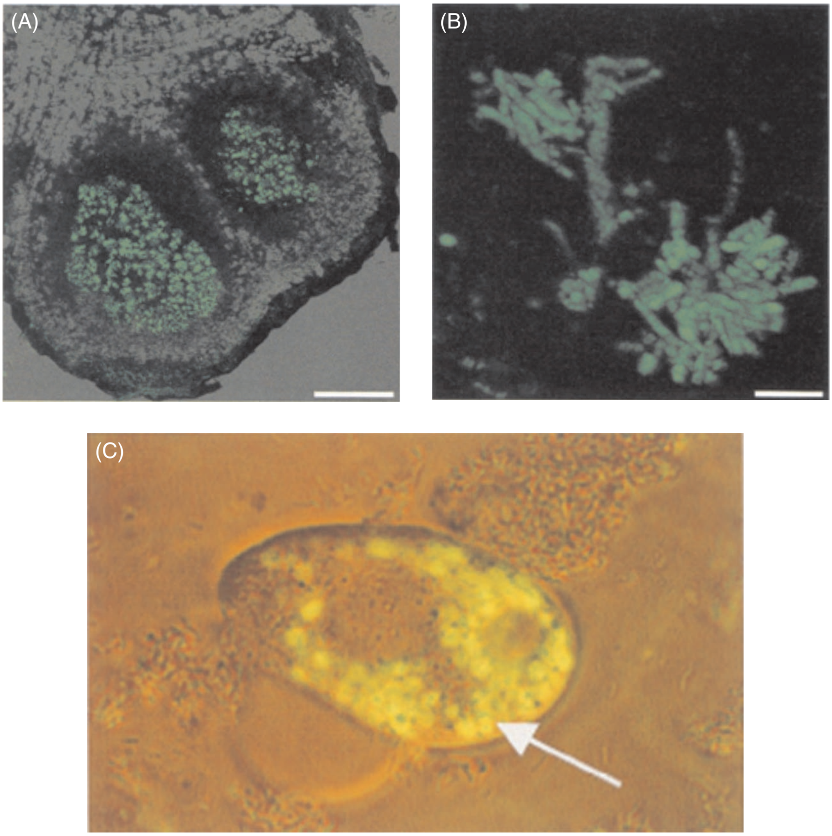



FIGURE 4.5 (A and B) The $N_2$-fixing rhizobium *Sinorhizobium fredii* HH103, carrying the plasmid construct pMP4516 (ECFP) inside the nodule tissue of the legume host, Siratro (Stuurman *et al.,* 2000). (C) The protozoan *Tetrahymena* sp. after ingesting *gfp*-tagged *Moraxelia* sp. G21 cells (Errampalli *et al.*, 1999).

from soils of varying texture have been developed and these have been summarized by Bruns and Buckley (2002). There are two main approaches to nucleic acid extraction: (i) cell fractionation and (ii) direct lysis. In cell fractionation, intact microbial cells are extracted from the soil matrix. After extraction, the cells are chemically lysed and the DNA is separated from the cell wall debris and other cell contents by a series of precipitation, binding, and elution steps. In the direct lysis methods, microbial cells are lysed directly in the soil or sediment and then the nucleic acids are separated from the soil matrix by means similar to those described above. The main concerns when choosing a suitable protocol are extraction efficiency, obtaining a sample that is representative of the resident community, and obtaining an extract free of contaminants that could interfere with subsequent analyses such as PCR or hybridization with nucleic acid probes.

Extraction efficiency is a key concern for obtaining a nucleic acid extract that is representative of the soil community. Cell walls of different organisms are more or less amenable to lysis. Gram-positive organisms, resting spores and hyphae (fungi), and cysts (protozoa and nematodes) are more difficult to lyse than cells of Gram-negative organisms or vegetative stages of various soil fauna. Hence, unless lysis procedures are robust, a nucleic acid extract from soil may be biased toward organisms that are more readily ruptured. Extraction efficiency of both cell fractionation and direct lysis procedures can be assessed by direct microscopy, wherein extracted soil is examined for intact microbial cells using fluorescent stains. Alternatively, for assessing recovery efficiency using direct lysis procedures, soil samples may be spiked with a known quantity of labeled DNA and then the recovery of added DNA is assessed.

Obtaining a sample that is representative of the resident community is often challenging. Microbes in soil are frequently in a resting state or near starvation, making them more difficult to lyse than cells growing rapidly in culture media. Direct cell extraction protocols must ensure that cells are released from soil without bias and direct lysis procedures must ensure that nucleic acids are not adsorbed to clays or soil organic matter and thus not recovered. Failure to recover a representative sample of nucleic acids from soil is a potentially significant source of bias that may affect later data interpretation.

Coextraction of contaminants, such as humic substances, is also a common problem. Such contaminants may interfere with PCR amplification or hybridization experiments to the point where the reactions may fail entirely. Several methods have been suggested to eliminate or reduce contaminants. One approach is to use a commercial post-PCR DNA clean-up kit. While these kits are normally designed to remove salts from post-PCR amplification reactions, they may also work to reduce contaminants in DNA extracts, thereby reducing PCR inhibition in many instances. An alternative method is to subject the nucleic acid extract to an additional washing step with dilute EDTA, pass the extract through a Sephadex G-75 column, or gel purify the nucleic acid prior to analysis. In many cases, a simple dilution of the DNA extract may alleviate problems with PCR amplification, but may also dilute out soil community members of interest. It must be kept in mind that all manipulations of nucleic acid extracts can lead to loss of material and hence sparsely represented members of the community may be lost from subsequent analyses. In addition, all postextraction clean-up procedures add extra expense and processing time, thus reducing the number of samples that can be analyzed within the scope of any experiment.

Commercial soil DNA and RNA extraction kits that are based on direct lysis by bead-beating have become available. The DNA/RNA extracted is of high molecular weight and usually of sufficient quality to be used directly in PCR or nucleic acid hybridization experiments for most soils.

Many postextraction analysis procedures require that the concentration of extracted nucleic acids and their quality are known. Several methods, including fluorimetry with use of Hoechst 33258 or SYBR Green I dye, in which absorbance

is recorded and compared to a calf thymus DNA standard curve, or simply gel electrophoresis against standard, commercially available, *Escherichia coli* DNA, can be used to estimate nucleic acid concentrations. A UV spectrophotometer allows the absorbance of the extract at 260 (DNA) and 280 nm (contaminating proteins) to be measured. The ratio between these two readings is an indicator of the purity (quality) of the DNA extract.

## CHOOSING BETWEEN DNA AND RNA FOR SOIL ECOLOGY STUDIES

A key decision a researcher must make prior to molecular analysis of soil microbial communities is whether to extract microbial DNA, RNA, or both types of nucleic acids. DNA analysis has been used most frequently because DNA is more stable and easier and less costly to extract from soil. Postextraction analyses are straightforward and yield considerable information about the presence of various organisms in a given sample. The key problem with DNA analysis is that it does not reflect the abundance or level of activity of different organisms in a sample. When cells die, DNA released into the soil solution is rapidly hydrolyzed by nucleases. However, DNA contained in dead cells within soil aggregates or otherwise protected from decomposition will be extracted along with that from moribund and active cells. RNA on the other hand is highly labile and often difficult to extract from soil. In practice, only rRNA can be extracted with reasonable efficiency from soil at this time. A relatively simple method for ribosome extraction from soil is given in Felske *et al.* (1996) and commercial extraction kits are also available. Extraction of mRNA, which could be used to examine gene expression in soil under varying conditions, has been impossible until very recently. Even now, it is fraught with difficulty as mRNA is often extremely short-lived and is frequently being transcribed and translated simultaneously (see Fig. 4.2) in prokaryotes. Many postextraction analyses require that RNA is first reverse-transcribed into cDNA and then the cDNA used in downstream analyses. The advantage of extracting and analyzing RNA is that it is generally present in high amounts only in actively metabolizing cells. As substrate becomes limiting, cell processes slow down and, in some organisms, rRNA turnover may also be slowed. Thus, analysis of RNA is more reflective of the portion of the soil microbial community that is active at the time of sampling or has recently been active.

## ANALYSIS OF NUCLEIC ACID EXTRACTS

### DNA:DNA REASSOCIATION KINETICS

When DNA is denatured by either heating or use of a denaturant (e.g., urea), the double helix structure is lost as the two strands, held by hydrogen bonds between complementary base pairs (A:T and G:C), come apart. When the denaturant is removed or the temperature is lowered, complementary strands will reanneal (Fig. 4.6). When genome complexity is low, the time it takes for all single strands to find

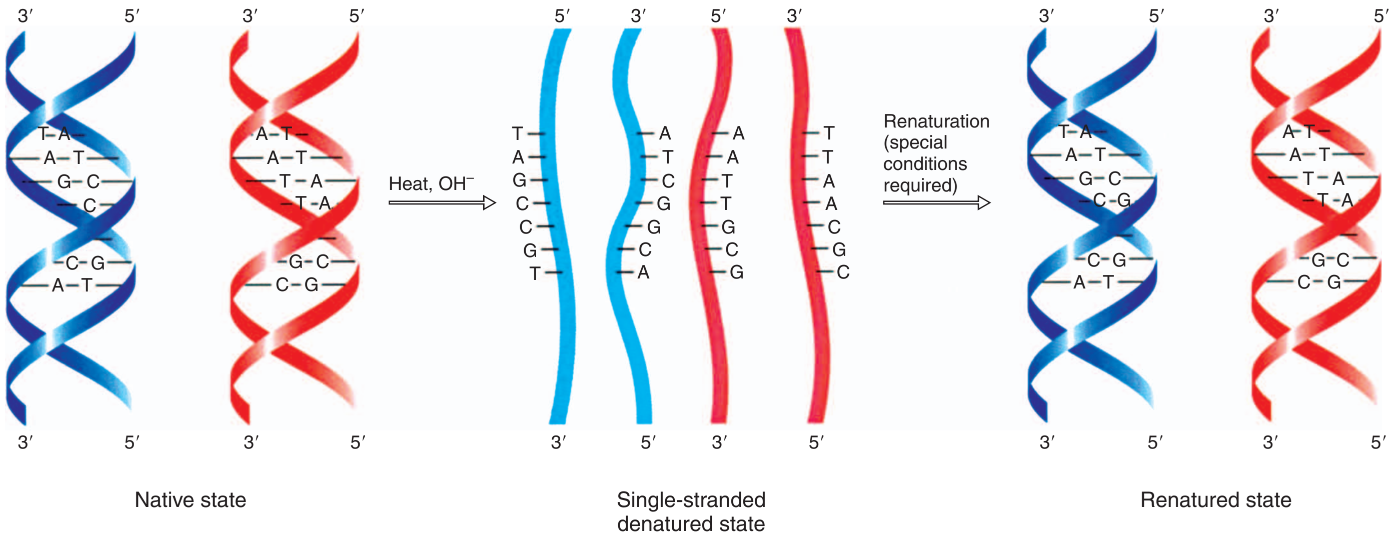



FIGURE 4.6 DNA:DNA hybridization. Native double-stranded DNA is denatured using heat or alkali into single strands. Under proper conditions, single strands will reanneal with strands having the complementary sequence, creating hybridized double-stranded DNA.

their complement is brief. As complexity increases, the time it takes for complementary strands to reanneal increases. Experimentally, this is referred to as a $C_o t$ curve, where $C_o$ is the initial molar concentration of nucleotides in single-stranded DNA and $t$ is time. This measure reflects both the total amount of information in the system (richness or number of unique genomes) and the distribution of that information (evenness or the relative abundance of each unique genome) (Liu and Stahl, 2002), thus making it among the more robust methods for estimating extant diversity in a given sample. Yet, it provides no information on identity or function of any member of the microbial community.

The genetic complexity or genome size of several soil microbial communities was assessed using reassociation kinetics by Torsvik *et al.* (1990; 1998). Using this procedure, they estimated that the community genome size in undisturbed organic soils was equivalent to 6000–10,000 *E. coli* genomes. In comparison, a heavy-metal-polluted soil contained 350–1500 genome equivalents. Culturing produced fewer than 40 genome equivalents. Such experiments, and those employing direct counts using epifluorescence microscopy, are what substantiate the lack of abundance and diversity commonly observed using culturing methods. The data from the above study were reanalyzed using improved analytical methods, which yielded an estimate of the extant diversity contained in the undisturbed soil sample of 8.3 million distinct genomes in 30 g of soil, two orders of magnitude greater than originally derived (Gans *et al.*, 2005). In contrast, the heavy-metal-polluted soil was estimated to contain only 7900 genome equivalents, 99.9% fewer than in the undisturbed soil.

## MICROARRAYS

Microarrays represent an exciting new development in microbial community analysis. Nucleic acid hybridization is the principle on which the technique is based. The main difference between past protocols and microarrays is that the oligonucleotide probes, rather than the extracted DNA or RNA targets, are immobilized on a solid surface in a miniaturized matrix. Thus, thousands of probes can be tested for hybridization with sample DNA or RNA simultaneously. In contrast to other hybridization techniques, the sample nucleic acids to be probed are fluorescently labeled, rather than the probes themselves. After the labeled sample nucleic acids are hybridized to the probes contained on the microarray, positive signals are detected by use of CSLM or other laser microarray scanning device (Fig. 4.7). A fully developed DNA microarray could include a set of probes encompassing virtually all known natural microbial groupings and thereby serve to monitor the population structure simultaneously at multiple levels of resolution (see Table 4.1; Guschin *et al.*, 1997; Ekins and Chu, 1999; Wu *et al.*, 2001). Such an array would potentially allow for an enormous increase in sample throughput. A major drawback of microarrays for use in soil ecology studies currently is their need for a high copy number of target DNA/RNA to obtain a signal that is detectable with current technologies. Targets in concentrations of less than $10^3$–$10^4$ are difficult to detect

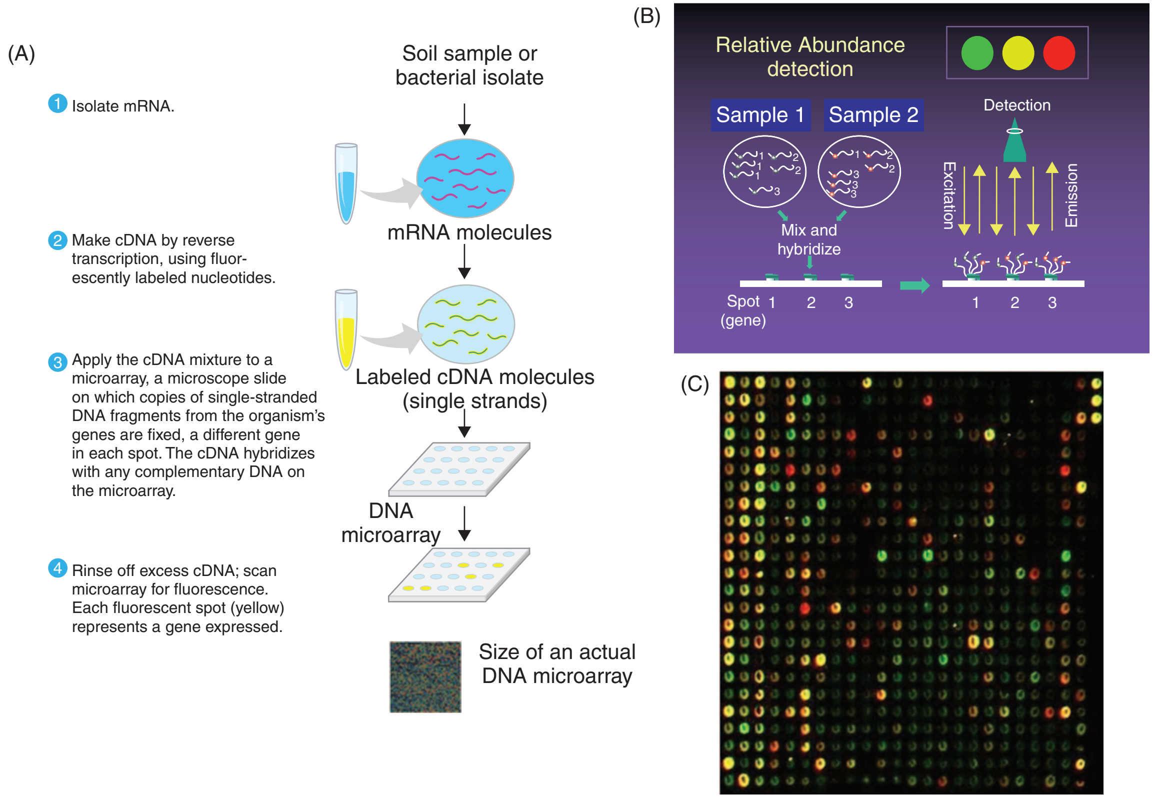



FIGURE 4.7 Steps in developing and using a microarray. (A) Flow chart depicting the steps from extracting mRNA from a sample to reading the microarray with a laser scanner (Campbell and Reece, 2005). (B) Detail of how the labeled cDNA interacts with the probes bound to the microarray and how a positive signal from one or the other sample is detected. (C) An image of a microarray reading (http://www.bsi.vt.edu/ralscher/gridit/).

using this approach. Techniques to improve the sensitivity have been reported by Denef *et al.* (2003). Nonspecific binding of target nucleic acids to the probes is also a serious issue that needs to be overcome (Zhou and Thompson, 2002). The details of microarray construction and types of arrays can be found in Ekins and Chu (1999) and ecological applications are reviewed in Zhou (2003).

There are three basic types of arrays used in soil ecology: (i) community genome arrays (CGA), used to compare the genomes of specific groups of organisms; (ii) functional gene arrays (FGA), used to detect the presence of genes of known function in microbial populations in prepared soil samples and more recently used to detect gene expression; and (iii) phylogenetic oligonucleotide arrays (POA), used to characterize the relative diversity of organisms in a sample through the use of rRNA sequence-based probes. Whole genomic DNA, requiring culturing of target organisms, is used to develop the probes used in CGA. Both oligonucleotides and DNA fragments derived from functional genes, such as those involved in C, N, S, and metal cycling, can be used to prepare FGAs and query the status of these functional genes within a soil community. In POA, both conserved- and variable-region rRNA gene sequences are used to determine the presence of particular phylogenetic groups in a given sample.

## RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP) ANALYSIS

In RFLP analysis, total DNA purified from soil is hydrolyzed with a restriction endonuclease (often *Eco*RI or *Hin*dIII). Restriction enzymes, produced by and originally isolated from various bacteria, cut double-stranded DNA at palindromic sequences (those that read in the same order both backward and forward). Restriction enzymes can be selected that cut either frequently or infrequently along the isolated DNA strands. The variation (polymorphisms) in the length of resulting DNA fragments is visualized by running the DNA fragments on an electrophoretic gel and staining the gel with ethidium bromide or SYBR Green I, which fluoresce under UV light. These variations in fragment lengths are then used as a “fingerprint,” to differentiate between soil communities. Discrimination between communities is often difficult because of the large number of fragments generated and the difficulty in resolving closely spaced bands in a gel. RFLP is rarely used on its own for diversity studies. It is used most commonly in conjunction with the Southern method of transferring (blotting) the DNA fragments from the gel onto a nitrocellulose or other membrane and then probing the blot with appropriately labeled oligonucleotide gene probes to test for the presence of specific sequences or used in conjunction with PCR of amplified ribosomal genes in a technique called ARDRA (amplified ribosomal DNA restriction analysis). The ultimate aim of RFLP-based methods is to be able to compare differences between DNA fingerprints obtained from different communities. Observed differences can then be characterized more fully using other methods, largely involving cloning and sequencing of DNA fragments of interest.

## CLONING

DNA sequence information is obtained from environmental samples in two main ways, (i) cloning DNA extracted from soil directly or (ii) cloning of PCR-amplified DNA, followed in both cases by sequencing of the cloned DNA. Direct cloning involves isolating DNA from the soil, ligating the DNA into a vector (most frequently a self-replicating plasmid), and transforming (moving) the vector into a competent host bacterium, such as commercially available *E. coli* competent cells, where it can be maintained and multiplied. In this way, a recombinant DNA clone library is produced. Once a clone library is obtained, DNA inserts contained in the clones can be reisolated from the host cells, purified, and sequenced. The clone library can also be screened for biological activity expressed directly in *E. coli* or probed for sequences of interest using various genomics applications. This approach circumvents the need to culture microorganisms from environmental samples, and it also provides a relatively unbiased sampling of the genetic diversity of sampled environments.

It has become possible to clone large fragments (100–300 kb) of DNA into bacterial artificial chromosome (BAC) vectors (Rondon *et al.*, 2000). BAC vectors are low-copy-number plasmids that can readily maintain large DNA inserts. When some of the BAC libraries were analyzed, sequences homologous to the low-G + C, Gram-positive *Acidobacterium, Cytophagales*, and *Proteobacteria* were found. Rondon *et al.* (2000) also identified clones that expressed lipase, amylase, nuclease, and hemolytic activities. Hence, the library could be used both for phylogenetic studies and as a tool for natural product discovery. Probing metagenomic libraries from a range of environments led to the discovery that uncultivated members of the archaeal lineage Crenarchaeota contain gene sequences with homology to the ammonia monooxygenase genes in nitrifying bacteria (Schleper *et al.*, 2005). This discovery suggests that the Crenarchaeota may be playing a more significant role in the global cycling of N than thought previously (Schleper *et al.*, 2005; Nicol and Schleper, 2006). Such metagenomic libraries are powerful tools for exploring soil microbial diversity and will form the basis of future genomic studies that link phylogenetic information with soil microbial function.

An alternative approach for creating large clone libraries from soil sequences that allows subsequent profiling of microbial communities is called serial analysis of ribosomal sequence tags (SARST). In this approach, a region of the 16S rRNA gene is amplified by PCR, such as the V1 region (variable in sequence between taxonomic groups). Through a series of enzymatic and ligation (linking) steps, the various V1 region amplicons are joined together. The resulting concatemers are then purified, cloned, screened, and sequenced. The sequences of the individual V1 amplicons are deduced by ignoring the linking sequences and analyzing each sequence tag individually (Neufeld *et al.*, 2004). Several other PCR-based community analysis methods (see Partial Community Analyses—PCR-Based Assays) allow DNA fragments to be retrieved in a selective manner and these can then be cloned using the methods described above.

## DNA SEQUENCING

Significant progress, particularly in working with environmental samples, has been made since the dideoxy chain termination method for DNA sequencing was first described by Sanger *et al.* (1977). The advent of fluorescent dyes, improvements in gel matrix technology, cycle sequencing using a thermal cycler, capillary systems, use of lasers, and automated gel analysis now allows up to 1100 bases of sequence to be generated or deduced in a single reaction. Manual sequencing is extremely time-consuming and is very rarely performed in individual laboratories any more. It is more cost effective to send sample DNA to a commercial sequencing facility for analysis.

Part of the usefulness of DNA sequencing lies in determining gene sequences of unique or important members of a soil community for use in developing more specific primers and gene probes to address specific ecological questions. Gene sequences, once obtained, are submitted to and maintained within various databases such as GenBank (http://www.ncbi.nlm.nih.gov/) or the Ribosomal Database Project II (http://rdp.cme.msu.edu/html/). GenBank and its collaborating databases, the European Molecular Biology Laboratory (EMBL; http://www.ebi.ac.uk/embl/) and the DNA Databank of Japan (DDBJ) reached a milestone recently of containing 100 billion bases (100 gigabases) of sequence information from over 165,000 organisms, including bacteria, fungi, protozoa, nematodes, and other fauna. The Ribosomal Database Project II, Release 9.35 (Cole *et al.*, 2005), contains nearly 198,000 aligned and annotated bacterial small subunit (16S) rRNA gene sequences with updated online analyses. Continued development of databases through DNA sequencing is essential and is a prerequisite to good primer and probe design.

## STABLE ISOTOPE PROBING

Among the more exciting advances in molecular ecology is the use of stable isotope probing (SIP). This approach allows microbial identity to be linked to functional activity through the use of substrates labeled with stable isotopes. It has been used to its best advantage by labeling substrates that are used almost exclusively by the population of interest (Radajewski *et al.*, 2000; Wellington *et al.*, 2003; Dumont and Murrell, 2005). In this method, a selected substrate is first highly enriched with a stable isotope, frequently $^{13}C$ or $^{15}N$, and then incorporated into the soil. After a brief incubation, cellular components of interest are recovered from the soil sample and analyzed for the incorporated stable isotope label. In this way, microbes that are actively using the added substrate can be identified. DNA and rRNA are the biomarkers used most frequently (Radajewski *et al.*, 2003), although PLFAs have also been used successfully (Treonis *et al.*, 2004). The isotopically labeled nucleic acids are purified from unlabeled nucleic acids by density-gradient centrifugation. Once separated, labeled nucleic acids can be amplified using PCR and universal primers to Bacteria, Archaea, or Eucarya. Analysis of the PCR products, through

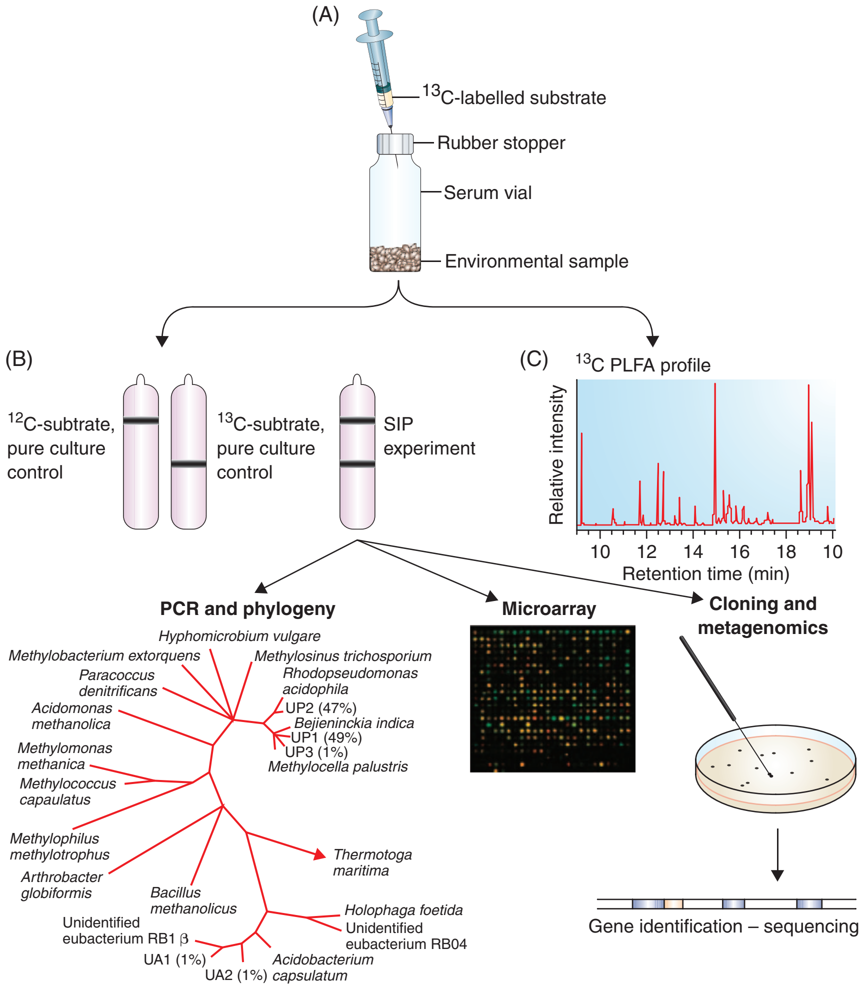



FIGURE 4.8 Stable isotope probing procedure with associated assays for assessing incorporation of isotopically labeled substrate into cellular constituents and determining the sequence(s) of bacteria that have incorporated the label (Dumont and Murrell, 2005).

cloning and sequencing for example, allows the microbes that have assimilated the labeled substrate to be identified (Fig. 4.8).

This approach has been applied successfully to study methanotrophs and methylotrophs (McDonald *et al.*, 2005) and active rhizosphere communities through $^{13}C–CO_2$ labeling of host plants (Griffiths *et al.*, 2004). In the latter approach, information about which microbes are assimilating root exudates under a given set of environmental conditions can be obtained. Rangel-Castro *et al.* (2005) used $^{13}C–CO_2$ pulse labeling, followed by RNA-SIP, to study the effects of liming on the structure of the rhizosphere microbial community metabolizing root

exudates in a grassland. Their results indicated that limed soils contained a microbial community that was more complex and more active in using $^{13}C$-labeled compounds in root exudates than were those in unlimed soils. SIP-based approaches hold great potential for linking microbial identity with function (Dumont and Murrell, 2005), but at present a high degree of labeling is necessary to be able to separate labeled from unlabeled marker molecules. This need for high substrate concentrations may bias community responses. Alternatively, use of long incubation times to ensure that sufficient label is incorporated increases the risk of having cross-feeding of $^{13}C$ from the primary consumers to the rest of the community, complicating data interpretation. Another complicating factor is identifying enriched nucleic acids within the density gradient. The point at which a given nucleic acid molecule is retrieved from the CsCl gradient is a function of both the incorporation of the heavy isotope and the overall G + C content of the nucleic acids. Thus, a means to attribute band position in the gradient to either label incorporation or high G + C content must be devised.

## PARTIAL COMMUNITY ANALYSES—PCR-BASED ASSAYS

PCR involves the separation of a double-stranded DNA template into two strands (denaturation), the hybridization (annealing) of oligonucleotide primers (short strands of nucleotides of a known sequence) to the template DNA, and then the elongation of the primer–template hybrid by a DNA polymerase enzyme. During PCR, each of these steps is accomplished by regulating the heat of the reaction. The temperature is raised to 92–96°C to denature the template DNA and then lowered to 42–65°C to allow the primers to anneal to the template. The temperature is then raised to 72°C or the ideal temperature for the activity of the DNA polymerase used in the reaction. This cycle is repeated from 25 to 30 times, each cycle doubling the number of products (amplicons) in the reaction (Fig. 4.9). The discovery of thermal-stable DNA polymerases from organisms such as *Thermus aquaticus* (*Taq* polymerase) has made PCR possible as a standard protocol in laboratories around the world (Mullis and Faloona, 1987; Saiki *et al.*, 1988) and led to the award of a Nobel Prize to Kary Mullis in 1993.

The temperatures chosen at each step in the thermal cycling are specific to each protocol. The annealing step is a critical choice. Lower temperatures are less stringent and may allow base "mismatch" to occur when the primer binds to the template. Higher annealing temperatures are more stringent and therefore primers bind with higher fidelity to their target sequences. The potential target genes for PCR are many and varied, limited only by available sequence information. The primers used for soil ecological studies may target specific DNA sequences, such as those coding for the small subunit (SSU) rRNA genes; sequences of genes of known function; or sequences that are repeated within microbial genomes (rep-PCR); or arbitrary primers may be used to generate a PCR "fingerprint."

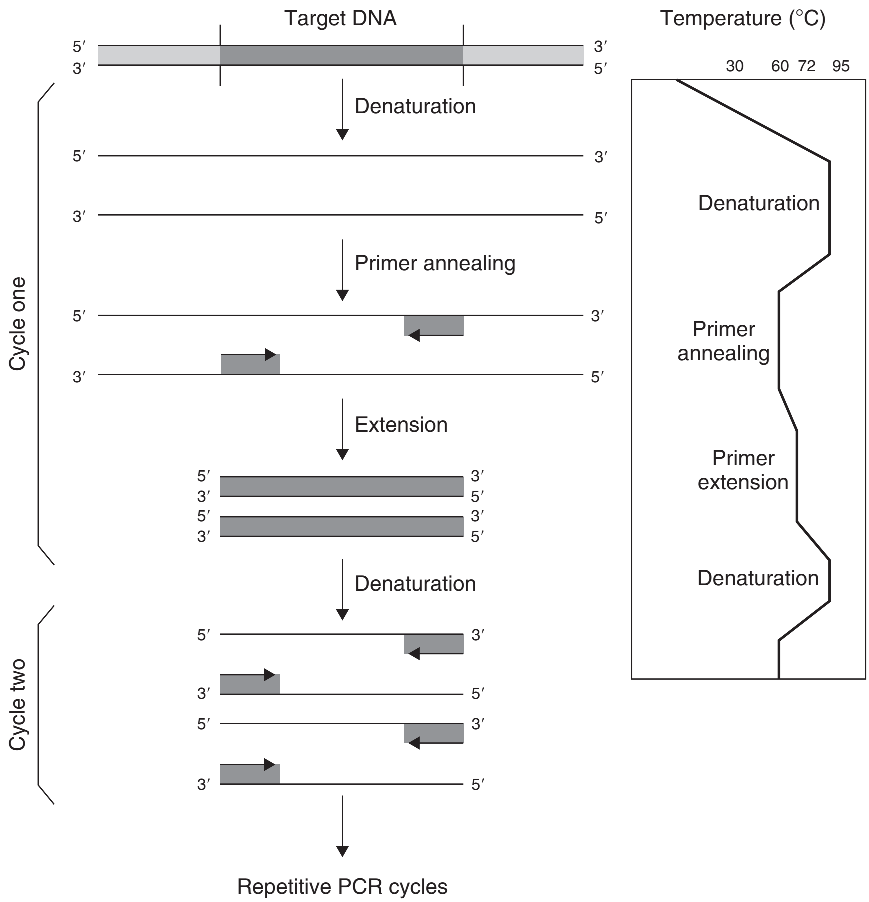



FIGURE 4.9 Mechanics of the polymerase chain reaction. The right depicts the temperature variation occurring in a thermal cycler during the reaction. The left illustrates how the DNA template and primers interact to copy the target DNA during the changes in temperature as cycling proceeds.

Primers can be selected to target different levels of taxonomic resolution. Ribosomal RNA genes are highly conserved and therefore discriminate between sequences at the genus level or above. Repeat-sequence and arbitrary primers are used to discriminate at a finer scale, separating isolates at the strain level. Several oligonucleotides used commonly in PCR fingerprinting and their level of resolution are listed in Table 4.3. While small subunit rRNA genes are used successfully in community analysis, they are able to resolve bacterial and archaeal groups only at higher levels of taxonomic classification.

By far the more common targets for characterizing microbial communities are the rRNA genes because of their importance in establishing phylogenetic and taxonomic relationships (Woese *et al.*, 1990). These are the SSU rRNA genes, 16S

TABLE 4.3 Examples of Primers Used Commonly for Amplifying DNA Extracted from Soil or to Characterize Soil Bacterial Isolates

| Name | Target gene | Target | Reference |
|---|---|---|---|
| 27F-1392R | 16S rRNA | Small subunit ribosomal RNA gene | Lane, 1991 |
| 1490F-132R | ITS between 16S and 23S rRNA | Internal transcribed spacer | Navarro *et al.*, 1992; Ponsonnet and Nesme, 1994 |
| *amoA*-1F-*amoA*-2R | *amo*A | Ammonia monooxygenase | Rotthauwe *et al.*, 1997 |
| PolF-PolR | *nif*H | Dinitrogenase reductase | Poly *et al.*, 2001 |
| A189-A682 | *pmo*A | Particulate methane monooxygenase | Horz *et al.*, 2001 |
| ERIC 1R-ERIC 2 | Repeated elements | Enterobacterial repetitive intergenic consensus sequences | Versalovic *et al.*, 1991 |
| REP1R-REP 2 | Repeated elements | Repetitive extragenic palindromic sequences | Versalovic *et al.*, 1994 |

in Bacteria and Archaea or 18S in Eucarya; the large subunit (LSU) rRNA genes, 23S in Bacteria and Archaea or 28S in Eucarya; or the internal transcribed spacer (ITS) regions, sequences that lie between the SSU and the LSU genes. Other defined targets are genes that code for ecologically significant functions, such as genes that code for proteins involved in nitrogen fixation, e.g., *nif* H, which encodes the iron protein of nitrogenase reductase; *amo*A, which codes for ammonium monooxygenase, a key enzyme in nitrification reactions; and *nir*S, which codes for nitrite reductase, a key enzyme in denitrification reactions.

In any study in which PCR is used, sources of bias must be considered (v. Wintzingerode *et al.*, 1997). The main sources of bias in amplifying soil community DNA are: (i) the use of very small sample sizes (typically only 500 mg of soil), which may represent only a small fraction of the whole soil community; (ii) preferential amplification of some DNA templates over others due to the greater ease of binding of DNA polymerase to some sequences over others; and (iii) for amplification of the rRNA genes, the fact that many bacteria contain multiple copies of these operons (e.g., *Bacillus* and *Clostridium* species contain 15 copies), hence sequences from such species will be overrepresented among the amplification products. In addition, chimeras, composed of double-stranded DNA in which each strand was derived from a different organism rather than a single organism, may be generated. This latter problem is sometimes a consequence of using too many cycles in the PCR. Acknowledged biases associated with PCR are generally why diversity indices calculated from the results of PCR-based experiments may not be very robust and their use is not encouraged.

An advance in PCR analysis that allows specific gene targets to be quantified is quantitative PCR (qPCR), also called real-time PCR. qPCR is a method that employs fluorogenic probes or dyes to quantify the number of copies of a target DNA sequence in a sample. This approach has been used successfully to quantify

target genes that reflect the capacity of soil bacteria to perform given functions. Examples include the use of ammonia monooxygenase (*amo*A), nitrite reductase (*nir*S or *nir*K), and particulate methane monooxygenase (*pmo*A) genes to quantify ammonia-oxidizing (Hermansson and Lindgren, 2001), denitrifying (Henry *et al.*, 2004), and methanotrophic (Kolb *et al.*, 2003) bacteria, respectively, in soil samples. qPCR coupled with primers to specific ITS or rRNA gene sequences has also been used to quantify ectomycorrhizal (Landeweert *et al.*, 2003) and endomycorrhizal fungi (Filion *et al.*, 2003) as well as cyst nematodes (Madani *et al.*, 2005) in soil.

## ELECTROPHORESIS OF NUCLEIC ACIDS

Amplified PCR products are visualized most often by running samples in an electrophoretic gel; staining the DNA within the gel with ethidium bromide, SYBR Green I, or another fluorescent dye with a high-affinity binding to DNA; and viewing the stained, separated PCR products under UV light. Nucleic acids are negatively charged and will run to the positive pole in an electric field. The gel matrix provides resistance to the movement of nucleic acids through it by virtue of the pore sizes within the gel, such that DNA fragments of smaller size will move through the matrix faster than those of a larger size. A standard molecular weight marker is typically run along with samples to enable the size of PCR products to be assigned during gel analysis. The analysis of the amplified products is based on the presence and pattern of DNA bands of various sizes contained in the gel matrix.

Agarose is the most popular medium for electrophoretic separation of medium- and large-sized nucleic acids. Agarose has a large working range, but poor resolution compared with polyacrylamide. Depending upon the agarose concentration used, nucleic acids between 0.1 and 70 kb in size can be separated. Polyacrylamide is the preferred matrix for separating proteins, single-stranded DNA fragments up to 2000 bases in length, or double-stranded DNA fragments of less than 1 kb. Polyacrylamide gels have excellent resolving power as they separate macromolecules on the basis of configuration in addition to the more commonly exploited characteristics of size, charge, and G + C content. This shape-dependent mobility forms the basis of a suite of techniques that exploit inter- and intrastrand nucleotide interactions and can be used to screen amplified DNA rapidly for very fine-scale sequence differences. These techniques include single-strand conformation polymorphism (Dewit and Klatser, 1994), denaturing (or temperature) gradient gel electrophoresis (DGGE or TGGE) (Muyzer and Smalla, 1998), and heteroduplex mobility assays (Espejo and Romero, 1998). Because the electrophoretic mobility of nucleic acids using these techniques is highly sequence dependent, these techniques are often used in studies of genetic diversity.

## PCR FINGERPRINTING

PCR fingerprinting can be accomplished by several different methods, all of which are aimed at distinguishing differences in the genetic makeup of microbial

populations from different samples. The advantage of these techniques is that they are rapid in comparison with sequencing methods, thus enabling high sample throughput, and can be used to target sequences that are phylogenetically or functionally significant. Depending on the primers chosen, PCR fingerprints can be used to distinguish between isolates at the strain level or to characterize target microbes at the community level. The more common PCR fingerprinting techniques in use today for characterizing soil microbial community composition are DGGE or TGGE (Muyzer and Smalla, 1998) and terminal restriction fragment length polymorphism (T-RFLP) analysis (Liu *et al.*, 1997; Clement *et al.*, 1998). Both techniques can be used to separate PCR products that are initially of a similar length by employing additional methods to separate the amplicons into a greater number of bands that are then used for community comparisons.

DGGE and TGGE are identical in principle (Fig. 4.10). Both techniques impose a parallel gradient of denaturing conditions along a polyacrylamide gel. Double-stranded DNA (dsDNA) PCR amplicons are loaded in wells at the top of the gel and, as the DNA migrates, the denaturing conditions of the gel gradually increase. In DGGE, the denaturant is typically urea; in TGGE it is temperature. Because native dsDNA is a compact structure, it migrates faster than partially denatured DNA. The sequence of a fragment determines the point in the gradient gel at which denaturation will start to retard mobility. Sequence affects duplex stability by both percentage G + C content and neighboring nucleotide interactions (e.g., GGA is more stable than GAG). The resulting gel yields a ladder of bands in each lane characteristic of the DNA extracted and amplified from the original sample. There is not a direct correspondence between bands in the DGGE gel and organism diversity, however. Sequences amplified from the DNA of different organisms may have similar melting properties in the presence of the denaturant and thus occupy the same band in the denaturing gel. DNA fragments cloned from different bands may yield as many different sequences as clones analyzed. Since there is not a one-to-one correspondence between bands and taxa, the bands are referred to as operational taxonomic units (OTUs). The OTUs form the basis of similarity and multivariate analyses of data derived from various soil communities.

While the power of DGGE and TGGE to detect PCR amplicon diversity within a single gel is high, the sensitivity of the technique to variations in experimental conditions makes comparisons between gels very difficult. These techniques are therefore of greatest use in a preliminary screening to aid recognition of sample diversity. The resolving power of these and other gel-based analyses is limited by the number of bands capable of "fitting" and being counted as individual bands on a single gel. In practice, no more than 100 distinct sequence types may be resolved despite the potential for single base-pair sensitivity. An important advantage that DGGE analysis has over T-RFLP (see below) is that PCR amplicons of interest that are resolved on a DGGE gel can be excised from the gel, reamplified, cloned, and sequenced, thereby obtaining taxonomic and/or phylogenetic information about amplifiable members of the soil community. For phylogenetic assignment of cloned sequences, variable regions within the SSU rRNA genes are amplified. An important

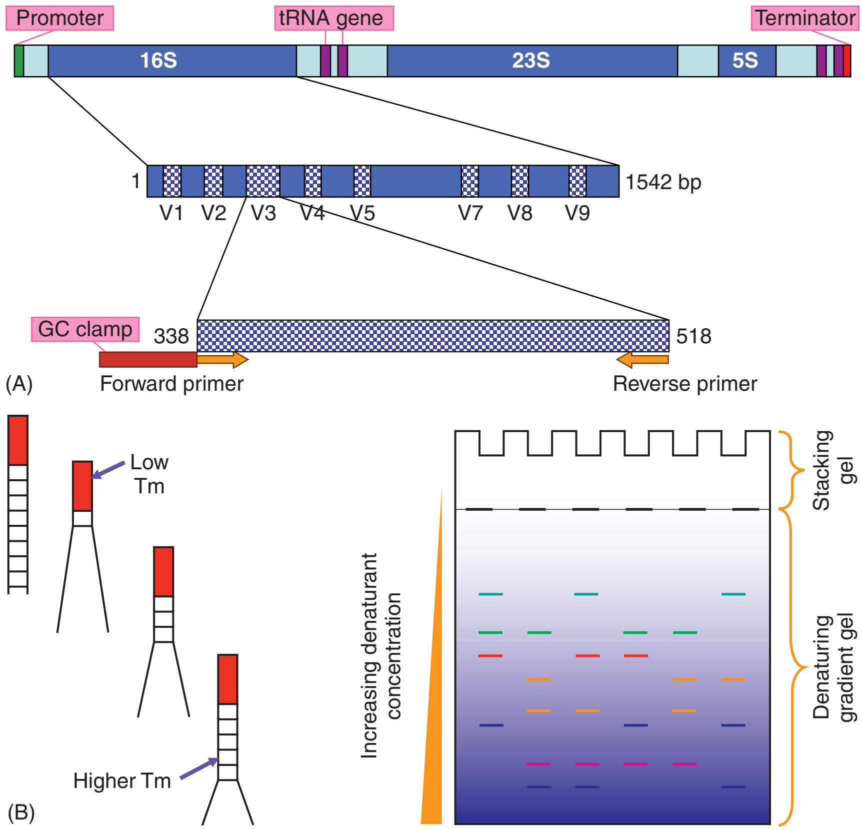



FIGURE 4.10 (A) Schematic representation of the denaturing gradient gel electrophoresis (DGGE) technique. In DGGE, the forward primer (f318) is tagged with a GC clamp to prevent the double strands from completely separating. Along with the reverse primer (r518), it amplifies the V3 region of the 16S rRNA gene in target DNA. (B) When amplified DNA products are loaded on a denaturing gradient gel and run in an electric field, the products separate according to their total ratio of A:T vs G:C base pairs and the locations of these base pairs relative to each other (with permission from R. Kantety, Alabama A&M).

disadvantage of the gradient gel approach is that the amplicon size must be restricted to under 600 bp in length to optimize separation within the gel matrix. Therefore, full-length rRNA gene sequences cannot be recovered using these methods. DGGE and TGGE are now being applied frequently in soil microbial ecology to compare the structures of complex microbial communities and to study their dynamics.

T-RFLP analysis, as in DGGE analysis, begins with amplifying soil community DNA using targeted primers, but with the key differences that one or both primers are labeled with a fluorochrome(s) and that resulting amplicons are cut with restriction enzymes to create DNA fragments of varying size, fluor-labeled at either the 5′ or the 3′ end (Fig. 4.11). These terminal fragments are then sized against

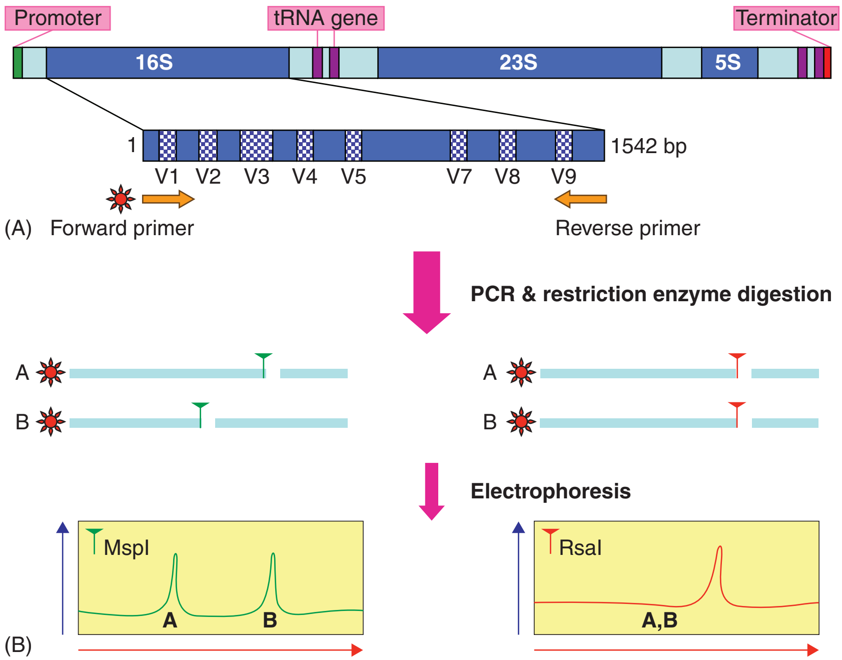



FIGURE 4.11 Schematic representation of the terminal restriction fragment length polymorphism (T-RFLP) technique. In T-RFLP, nearly the entire 16S rRNA gene is targeted with the primers f27 and r1492. The forward primer is tagged with a fluorochrome to facilitate length assignment of the terminal fragment after the amplified products are cut with a restriction endonuclease. The final step is to run the enzyme-digested products on a DNA sequencer, which will detect and report the lengths of all of the fluorochrome-labeled terminal fragments (with permission from R. Kantety, Alabama A&M).

a standard molecular size marker using automated DNA sequencing techniques. The resulting electropherogram (peaks representing the sizes of the terminal restriction fragments, TRFs) is used as a DNA fingerprint characteristic of the soil community sampled. Resulting TRF sizes are analogous to bands on a DGGE gel and are also referred to as OTUs, since any one terminal fragment size is not restricted to any taxonomic group per se (Liu *et al.*, 1997; Grüntzig *et al.*, 2002). TRF profiles are compared subsequently between samples by use of similarity matrices and multivariate statistics.

Although first described in 1997, there are now over 600 studies employing this technique of which over 100 report its use for the analysis of soil microbial communities. Liu *et al.* (1997) used T-RFLP to characterize microbial diversity within bioreactor sludge, aquifer sand, and termite guts. Other studies have applied the technique to compare rhizosphere microbial communities colonizing transgenic and nontransgenic plants (Devare *et al.*, 2004), the effects of soil management on fungal community composition (Edel-Hermann *et al.*, 2004), and the effects of heavy metals on bacterial communities (Turpeinen *et al.*, 2004),

TABLE 4.4 Comparison of the Number of Operational Taxonomic Units Generated by Four Different Molecular Community Analysis Methods for Each Sample[a] (Jones and Thies, in press)

| | | Method | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | DGGE (bands) | | T-RFLP (peaks) | | ARISA (peaks) | | 2D-PAGE (spots) | |
| Soil type | Sample | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| Low Zn | 1 | 23 | 22 | 83 | 93 | 115 | 131 | 294 | 289 |
| | 2 | 27 | 28 | 89 | 98 | 154 | 140 | 349 | 336 |
| Medium Zn | 1 | 23 | 29 | 62 | 61 | 151 | 146 | 356 | 359 |
| | 2 | 34 | 32 | 53 | 58 | 152 | 141 | 333 | 305 |
| High Zn | 1 | 38 | 37 | 60 | 76 | 126 | 131 | 257 | 266 |
| | 2 | 31 | 32 | 60 | 66 | 138 | 137 | 281 | 291 |

[a]Samples are divided into analytical replicates 1 and 2 for each method.

among many other applications. This technique need not be restricted to studying the 16S rRNA gene. T-RFLP can be used as a quick screen for any gene for which specific primers can be devised to look at differences between communities in environmental samples, such as *nif*H to compare populations of nitrogen-fixing bacteria or *amo*A to study ammonia-oxidizing bacterial populations in soil (Thies, in press). The main drawback of the use of this approach is the inability to characterize TRFs further or obtain sequence information, as the sample is lost shortly after it is sized. However, once profiles are compared, the original PCR products from samples of interest can be used for cloning and sequencing experiments as described above.

T-RFLP often yields a higher number of OTUs for use in comparative analyses than DGGE (Table 4.4). However, all of these techniques yield numbers of OTUs that do not come close to the estimates of extant diversity in soil populations as estimated by DNA:DNA reassociation kinetics (discussed previously). Hence, we are still viewing the tip of the iceberg as far as characterizing soil microbial diversity with these higher throughput DNA fingerprinting techniques.

A new approach, which combines gel separation of PCR amplicons of the ITS regions by size in a nondenaturing gel and then by melting characteristics in a second, denaturing gel has been developed (2D-PAGE; Jones and Thies, in press). This approach yields an order of magnitude greater number of OTUs than DGGE alone and three times the number of OTUs obtained by use of T-RFLP or automated ribosomal intergenic spacer analysis (ARISA) (Table 4.4). The disadvantage of this technique is that it is more laborious, therefore it does not lend itself to high sample throughput. Yet, its improved ability to discriminate between soil communities and retrieve sequence information make it a powerful technique for elucidating key differences in community structure between studied samples.

Several additional PCR fingerprinting techniques target the ribosomal gene sequences. Ribotyping makes use of differences in the chromosomal positions or structure of rRNA genes to identify or group isolates of a particular genus or species. Ribotyping has been shown to be reproducible and hence has gained popularity for isolate fingerprinting and has found use in bacterial source tracking and other studies in which the similarity of isolates obtained from different samples needs to be compared. The most frequently used ribotyping method is to identify RFLPs of rRNA genes by probing a Southern transfer of genomic DNA that has been hydrolyzed with an endonuclease. In ARDRA, rRNA gene sequences are amplified. In ARISA, the ITS region is amplified. PCR amplicons resulting from use of both methods are hydrolyzed subsequently with restriction enzymes and the resulting variations in restriction fragment sizes are analyzed on a gel. Bands in the gel are again termed OTUs and similarities and differences between the fingerprints from different samples are analyzed using multivariate techniques. Use of ARISA may yield more OTUs from a given sample, but as the number of bands on the gel increases, so does the difficulty one has in resolving individual bands in the analysis.

### SIMILARITY ANALYSES

The successful application of molecular techniques to population studies, particularly those based on the analysis of DNA or RNA in a gel matrix, relies heavily on the correct interpretation of the banding or spot patterns observed on electrophoretic gels. Gel images are typically digitized and band detection software is used to mark the band locations in the gel. The resulting band pattern is then exported to a statistical software package for analysis. Some analyses require that the fingerprint patterns obtained are first converted to presence/absence matrices; although average band density data are also used. The matrices generated are then compared using cluster analysis, multidimensional scaling, principal component analysis, redundancy analysis, canonical correspondence analysis, or additive main effects multiplicative interaction model, among others. Each analysis will allow community comparisons, yet each has associated strengths and weaknesses. There are a number of software packages available that will enable one to compare and score PCR fingerprints and produce similarity values for a given set of samples. Software packages, such as BioNumerics and GelCompar (Applied Maths, Kortrijk, Belgium), Canoco (Microcomputing, Ithaca, NY, USA), and PHYLIP (freeware via GenBank and the RDPII), among others, are used commonly.

## LEVEL OF RESOLUTION

Genes change as they acquire fixed mutations over time. The number of differences between two homologous sequences reflects both the evolutionary rate of the sequences and the time separating them, in other words—how long it has been

since they had a common ancestor. Consequently, different sequences need to be selected to resolve variation at different taxonomic levels. In general, noncoding DNA evolves faster than transcribed DNA, since it is under no selection pressure to remain unchanged; therefore, intergenic spacer regions evolve more rapidly than other sequences. Next is the "wobble" position of protein-coding genes, and slowest to change are the structural rRNA genes. The information that can be obtained from the use of molecular approaches depends on the analysis technique chosen. The level of resolution required, coupled with study aims, will largely guide the choice of technique used for a given study (Fig. 4.3).

## OTHER FACTORS THAT MAY AFFECT MOLECULAR ANALYSES

### SAMPLE HANDLING

Any study of soil microbial ecology requires that we consider the spatial and temporal interactions of potential reactants such as soil type and moisture content. Hence, the time, location, season of sampling, sample volume, mixing, compositing, and replication are all important for deriving meaningful data (see Chap. 3). Since all samples are by their nature small volumes on which measurements are taken to represent the whole soil, scaling errors can occur frequently. For molecular analyses, it is critical that representative samples are taken, that samples are protected from change between the field and the time they are analyzed in the laboratory, and that samples are not contaminated either by inadvertent mixing with each other or by coming in contact with other samples during handling, transport, and analysis. To avoid altering the composition of the microflora prior to analysis, soil samples should be stabilized as soon as practicable. For molecular measures, soil should be frozen immediately at –20°C and processed within several weeks of sampling. If analyses will be significantly delayed, soil should be stored at –80°C. Unlike sampling for soil chemical and physical parameters, soil drying should be avoided entirely when intended for analyzing microbial populations.

### SOIL CHEMICAL FACTORS

Among the key chemical factors that may interfere with molecular analysis of soil communities are soil constituents with cation and anion exchange capacity, such as humic acids, clays, and soil organic matter (SOM). Clay minerals and SOM possess a net negative charge. Individual, negatively charged clay particles in a moist soil will be surrounded by hydrated cations, which create a localized zone of positive charge. This will attract microorganisms, which possess a net negative charge at the pH of most soil habitats. Binding of bacteria to solid surfaces through such ionic interactions makes it difficult to separate both cells and DNA/RNA released from cells from the soil matrix. Soil type has a significant influence on

DNA/RNA extraction efficiency. Nucleic acid extraction may be particularly problematic from soils with high clay, organic matter, and/or humic contents. Effective removal of humic acids is often required prior to quantifying or amplifying DNA.

Humic substances inhibit *Taq* DNA polymerase in the PCR, interfere with restriction enzyme digestion, and reduce transformation efficiency during cloning and DNA hybridization specificity. Humic substances are difficult to remove as they remain soluble under conditions similar to those of DNA; hence, direct extraction of DNA may require an additional purification step to obtain DNA of sufficient purity for downstream assays. Use of polyvinylpyrrolidone may help to remove SOM from the cell preparations. Subsequent cesium chloride density gradient centrifugation yields DNA of high quality. Despite their effectiveness, these procedures are too labor intensive for use in large experiments. DNA extraction kits are available that include improved DNA clean-up steps and yield higher quality DNA extracts with fewer impurities that affect downstream DNA analyses.

The presence of humic acids and SOM also interferes with fluorescence microscopy. Nonspecific background fluorescence caused by binding of dyes to charged particles makes it difficult to resolve and quantify soil microorganisms. If microscopic images will be subject to image analysis, it also becomes difficult to "train" the software to separate cells from inorganic particles and produce an accurate count.

### SAMPLING SCALE

High spatial heterogeneity of soil influences the diversity of microbes and their function. Spatial heterogeneity in soil microbial communities occurs at many scales, from soil particles (e.g., soil microaggregates) to the plant rhizosphere, to field plots, and to the ecosystem and global levels. In order to improve our knowledge of how microbial community structure influences ecosystem function, we must obtain more quantitative knowledge of the interaction between microbes, plant residues, and soil management at a variety of spatial scales.

## SUMMARY

We have come a long way in developing our understanding of microbial ecology, but have many milestones yet to meet. Molecular tools offer unparalleled opportunities to characterize microbes in culture and directly from field soils. These tools are allowing us to ask questions at much larger geographic scales than has been possible previously. We are now able to examine such issues as how microbial populations vary across soil types and climatic zones, in association with plant roots and between various plant species, and in response to soil management or soil pollution. We are now just seeing the tip of the iceberg in terms of soil microbial diversity with the use of molecular approaches. The amount of work that remains is daunting, yet exciting as so much remains to be discovered. Recent techniques developed for the study of microbial populations, such as T-RFLP and

DGGE, allow access to the very large proportion of organisms that are present in the soil and which remain unculturable under laboratory conditions. Other techniques, such as RFLP analysis of isotopically labeled, amplified *nif*H, *amo*A, *nir*S, and *pmo*A sequences or SIP will allow us to target, with high specificity, organisms or groups of organisms responsible for specific functions in soil, particularly those involved in key transformations in the C, N, and S nutrient cycles. These types of technical developments open new horizons of research and applications that will allow a far more complete and less biased view of microbial diversity and function in soils.

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# 5

# The Prokaryotes

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Jim I. Prosser



## INTRODUCTION

Living organisms form three major domains: Bacteria and Archaea, collectively termed prokaryotes, and the Eucarya or eukaryotes. Eukaryotic soil organisms, including microorganisms, are discussed in Chaps. 6 and 7. Prokaryotes are distinguished from eukaryotes by the absence of a unit membrane-bound nucleus and, usually, the lack of other cell organelles. Ribosomes in prokaryotes are smaller (70S) than in eukaryotes (80S) and no eukaryote is able to fix atmospheric $N_2$. The endosymbiotic theory (Margulis, 1993) proposes that the mitochondria and chloroplasts of eukaryotic cells originated as symbiotic prokaryotic cells. The presence of bacterial, circular, covalently closed DNA and 70S ribosomes in mitochondria supports this theory. Despite the apparent, relative simplicity of prokaryotic cells, as a group they have the greater taxonomic and functional diversity. Globally, organic C in prokaryotes is equivalent to that in plants and they contain 10-fold

more N. They also possess the most efficient dispersal and survival mechanisms. As a consequence, prokaryotes are of enormous importance in creating, maintaining, and functioning of the soil. The aim of this chapter is to provide an overview of the taxonomy and characteristics of soil prokaryotes and show their importance both for prokaryote growth and activity and for soil function.

## PHYLOGENY

### CULTIVATED ORGANISMS

Historically, prokaryotes were classified on the basis of their phenotypic (observable) characteristics. Prokaryotic taxonomy therefore involved measuring a large number of characteristics, including morphology and biochemical characteristics (e.g., ability to grow on different substrates, cell wall structure, antibiotic sensitivities, and many others). This contrasts with classification of eukaryotic organisms, for which phylogenetic (evolution-based) classification was possible through the availability of fossil evidence.

A major revolution occurred with the realization that evolutionary relationships could be deduced on the basis of differences in gene sequence. The most important gene for prokaryote phylogeny is the 16S ribosomal RNA (rRNA) gene, which is present in all cells. The gene is approximately 1500 bases in length and possesses regions in which sequences are conserved, facilitating sequence alignment, and variable and hypervariable regions, which enable different organisms to be discriminated from one another. Genetic distance, calculated by quantitative comparison of sequence differences between organisms, allows evolutionary distance to be estimated.

A major discovery arising from this approach (Woese *et al.*, 1990) was that prokaryotes consist of two major domains, the Archaea and the Bacteria, which are as distant from each other as each is from the Eucarya (Fig. 5.1). The major

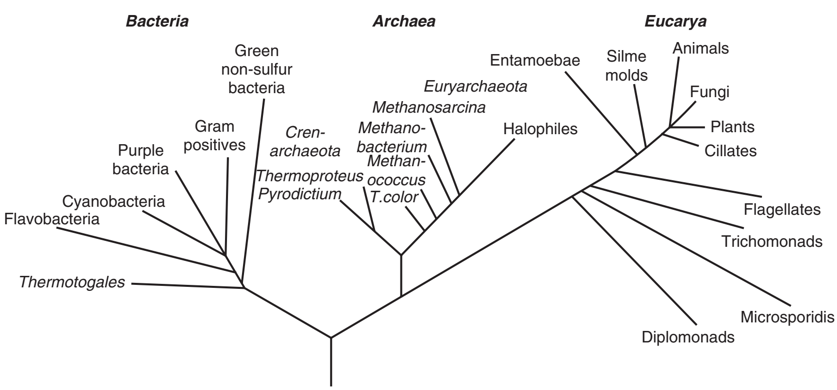



FIGURE 5.1 The universal tree of life constructed by analysis of sequences of small subunit rRNA genes (with permission from Wheelis *et al.*, 1992).

bacterial groups are shown in Figs. 5.1 and 5.2; the major archaeal groups are shown in Fig. 5.1. Each group in these domains is discussed in more detail below. This approach also led to a reanalysis and reappraisal of classification within the Archaea and Bacteria, and major divisions are presented in Tables 5.1 and 5.2. The 16S rRNA gene, while useful in defining high-level taxonomic groups, is less useful for fine-scale resolution. This requires analysis of other genes or alternative methods, e.g., DNA–DNA hybridization, comparison of sequences of several genes or of whole genomes, and comparison of proteins (see Chap. 4).

## UNCULTIVATED ORGANISMS

Molecular phylogeny was initially based on prokaryotes that could be cultivated in the laboratory, making 16S rRNA gene sequencing relatively easy. However,

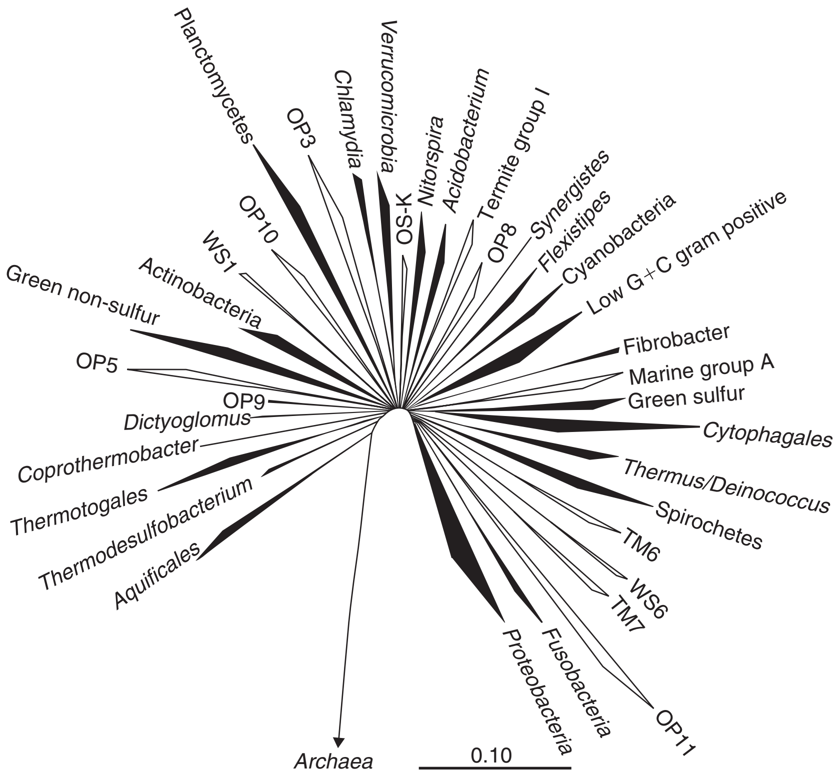



FIGURE 5.2 The major phylogenetic divisions within the Bacteria determined by analysis of 16S rRNA gene sequences. Unshaded segments represent groups with cultivated representatives, although many of these have very few such representatives. Shaded segments represent sequences obtained by direct amplification of 16S rRNA genes from environmental DNA, without a cultivation step (with permission from Hugenholtz *et al.*, 1998).

TABLE 5.1 Characteristics of Bacterial Phylogenetic Groups with Cultivated Representatives

| | Environmental origin | Metabolism | Other characteristics | Examples |
|---|---|---|---|---|
| Aquificales | Extreme environments (hot, sulfur pools, thermal vents) | Microaerophilic; chemolithotrophic; can oxidize hydrogen and reduced sulfur | | *Aquifex aeolicus* |
| Thermodesulfobacterium | Thermal vents | Sulfate reducers; autotrophic or organotrophic; anaerobic | Motile rods; gram-negative cell wall | *Thermodesulfobacterium hydrogeniphilum* |
| Thermotogales | Hot vents and springs; moderate pH and salinity | Sulfur reducers; organotrophic; some produce hydrogen | Prominent cell envelope | *Thermotoga maritima* |
| Coprothermobacter | Anaerobic digesters, cattle manure | Heterotrophic, methanogenic, sulfate reduction | Rod-shaped cells | *Coprothermobacter platensis* |
| Dictyoglomus | Hot environments | Chemoorganotrophic | Degrade xylan | *Dictyoglomus thermophilum* |
| Green nonsulfur bacteria and relatives | Wide range but few cultured | Anoxygenic photosynthesis (*Chloroflexus*); organotrophic (*Thermomicrobium*) | | *Chloroflexus*, *Herpetosiphon*, *Thermomicrobium roseum* |
| Actinobacteria (high G + C gram-positives, including actinomycetes) | Soil, some are pathogens | Aerobes, heterotrophic—major role in decomposition | Gram-positive, includes mycelial forms | |
| Planctomycetes | Soil and water | Obligate aerobes | Flagellated swarmer cells; budding bacteria, ovoid, holdfast, cell wall lacks murein | *Planctomyces, Pasteuria*, *Isocystis pallida* |
| Chlamydia | Intracellular parasites | Heterotrophic | No peptidoglycan | *Chlamydia psittaci*, *Trachomatis* |
| Verrucomicrobia | Freshwater and soil; few cultured | | | |
| Nitrospira | Soil and aquatic environments | Autotrophic nitrite oxidizers, facultative heterotrophs | Spiral shaped | *Nitrospira* |
| Acidobacterium | Wide range of environments, including soil | Acidophilic or anaerobic (very few cultured) | | |
| Synergistes | Anaerobic environments (termite guts, soil, anaerobic digesters) | Anaerobic | | |

| | | | | |
|---|---|---|---|---|
| Flexistipes | Animals | | Spiral shaped | |
| Cyanobacteria | Aquatic but found in soil | Oxygenic, photosynthetic; some fix $N_2$ | Gliding; unicellular, colonial or filamentous | *Aphanocapsa*, *Oscillatoria*, *Nostoc*, *Synechococcus*, *Gleobacter*, *Prochloron* |
| Firmicutes (low G + C gram-positive) | Soil, water, some are pathogens | Aerobic or anaerobic (rarely photosynthetic) | Cocci or rods; includes endospore formers | *Clostridium peptococcus*, *Bacillus*, *Mycoplasma* |
| Fibrobacter | | | | |
| Green sulfur bacteria | Anaerobic and sulfur-containing muds, fresh water and marine | Photosynthetic; anaerobic; autotrophic (S oxidation) or heterotrophic | Nonmotile | *Chlorobium*, *Chloroherpeton* |
| Bacteroides–Cytophaga–Flexibacter group | Wide variety, including soil, dung, decaying organic matter | Aerobic, microaerophilic or facultatively anaerobic, organotrophs, some strict anaerobes (*Bacteroides*) | Gliding (*Cytophaga*), gram-negative, rods, some pleomorphic, some helical, unbranched filaments | *Flavobacterium*, *Sphingobacterium*, *Cytophaga*, *Saprospira bacteroides*, *Prevotella*, *Porphyromonas* |
| Thermus/Deinococcus | High-temperature environments, nuclear waste | | Coccoid, rods; radioresistant or thermophilic; thick cell wall | *Deinococcus radiodurans*, *Thermus aquaticus* |
| Spirochetes and relatives (spirochetes and leptospiras) | Wide range | Chemoheterotrophic | Motile with flagellum; long, helical, coils; gram-negative | *Spirochaeta*, *Treponema*, *Borrelia*, *Leptospira*, *Leptonema* |
| Fusobacteria | Pathogens | Anaerobic | Gram-negative cell wall | |
| Proteobacteria | "Classical" gram-negative bacteria | Heterotrophs; chemolithotrophs; chemophototrophs; anaerobic (most) or aerobic; some photosynthetic; some fix $N_2$ | Often motile (flagella or gliding); gram-negative cell wall structure | *Rhizobacterium*, *Agrobacterium*, *Rickettsia*, *Nitrobacter*, *Pseudomonas*, *Nitrosomonas*, *Thiobacillus*, *Alcaligenes*, *Spirillum*, *Nitrosospira*, *Legionella* (some), *Beggiatoa*, *Desulfovibrio*, *Myxobacteria*, *Bdellovibrio* |

Some groups are represented by many cultivated organisms. In general, these are found in a wide range of habitats and exhibit a diversity of physiological and morphological characteristics. Other groups are represented by very few cultivated strains and the range of environments and physiologies listed above is likely to expand as additional environments are explored and isolation techniques improve.

TABLE 5.2 Characteristics of Archaeal Phylogenetic Groups

| | Environmental origin | Metabolism | Other characteristics | Examples |
|---|---|---|---|---|
| *Euryarchaeota* | | | | |
| Extreme halophiles | Salt lakes | Heterotrophic; aerobic; nonphotosynthetic phosphorylation | Require salt for growth, cell walls and enzymes stabilized by $Na^+$ | *Halobacterium*, *Natronobacterium* |
| Methanogens | Swamps, marshes, marine sediments, guts, sewage treatment | Anaerobic;generate methane; fix $CO_2$; electrons from hydrogen | | *Methanobacterium*, *Methanospirillum*, *Methanococcus* |
| Sulfur-metabolizing thermophiles | Hydrothermal vents | Anaerobic sulfur oxidizers, thermophiles, heterotrophs, methanogens, sulfate reducers | Polar flagella | *Thermococcus pyrococcus archaeoglobus* |
| *Crenarchaeota* | | | | |
| Hypothermophiles | Hot sulfur rich environments (hot springs, thermal vents) | Oxidize elemental sulfur, aerobic or anaerobic | Some lack cell wall, e.g., *Thermoplasma*; group includes *Thermus aquaticus* | *Thermoplasma sulfolobus*, *Acidothermus pyrodictium occultum* |
| Nonthermophilic crenarchaeotes | Soil, marine | One strain cultivated | | |
| Korarchaeota/ Xenarchaeota | Hot springs | None cultivated | | |
| Nanoarchaeota | Hot vents | Symbiont of Archaea | Coccoid; <400 nm in diameter | *Nanoarchaeum equitans* |

there was considerable evidence that numbers of organisms appearing on soil isolation plates were several orders of magnitude lower than total cell numbers, determined by microscopy and other methods (Torsvik *et al*., 1996). This suggested that knowledge of natural communities might be limited. The next revolution in microbial taxonomy confirmed this suspicion. It arose through the development of techniques for amplification, using the polymerase chain reaction, of 16S rRNA genes directly from DNA extracted from soil, without any intervening cultivation steps (Pace *et al*., 1986). Analysis of these sequences led to several major discoveries:

1. the existence of high-level, novel taxonomic groups with very few or no cultivated representatives;
2. high abundance of these groups in many soil environments;

3. tremendous diversity within taxonomic groups established on the basis of cultivated organisms;
4. the existence of novel subgroups within these established groups.

The outcome of molecular studies is illustrated in Fig. 5.2, which shows 40 high-level groups within the bacterial domain. Only ca. 50% of these groups have representatives in laboratory culture and representation is often low. Although not without disadvantages, molecular approaches have now replaced cultivation-based techniques for characterizing soil microbial communities (see Chap. 4). Their value has increased as sequence databases have expanded, which has enabled organisms to be putatively identified and compared between environments. Their use has also introduced new questions and challenges and has influenced our view of the ecology and role of soil microorganisms. For example, organisms that were previously considered to be "typical" soil organisms (bacilli, pseudomonads, actinobacteria) are often found at relatively low abundance, while some of the novel, "yet-to-be-cultured" organisms are ubiquitous and present at high relative abundance (e.g., planctomycetes; Rappe and Giovannoni, 2003). Similarly, Archaea were considered to be extremophiles, adapted to conditions atypical of most soils (high temperature, high salt concentration, acid, or anaerobic). It is now known that members of the Crenarchaeota typically represent 1–2% of temperate soil prokaryote communities, but have not yet been cultivated (Buckley and Schmidt, 2003). The lack of availability of cultivated representatives of these organisms denies us knowledge of their physiological characteristics and potential, and we can therefore only speculate on their role in soil. This has two implications for future studies—the need to develop methods for cultivating these organisms and/or the need to develop additional molecular approaches, or at least cultivation-independent approaches, to establish their ecosystem function *in situ.*

## PHYLOGENY AND FUNCTION

Molecular characterization of microbial communities would be of enormous value if the presence of a particular sequence or organism could be related unambiguously to its function in the soil. In some cases, phylogenetic groupings are informative. For example, all bacilli form resistant spores and the majority of rhizobia fix $N_2$. However, individual taxonomic groups can display considerable physiological versatility, and many functional characteristics are distributed among varied and evolutionarily distant groups. In addition, we know little of the physiological characteristics of novel groups and subgroups.

Even if cultivated representatives of all phylogenetic groups were available, a number of other issues must be considered for prokaryotes. The ordering and naming of hierarchical groups largely follow those for higher organisms, with the species as the basic unit of classification. For higher organisms, the species is defined through the Biological Species Concept and the ability of members within a species to interbreed and inability to breed with members of other species (Cohan, 2002). The lack of sexual reproduction mechanisms in prokaryotes prevents application of

this concept, with methodological and conceptual implications for our understanding of the development of distinct phylogenetic groups, evolution, and diversity. Prokaryotes can transfer genes by "lateral (or horizontal) gene transfer" (see Cell Structure), which bypasses standard evolutionary processes (mutation and selection), which can have an enormous influence on microbial community structure and activity. The most obvious example of this is the spread of plasmid-borne antibiotic resistance under selective pressure. Lateral gene transfer is not uncommon, particularly in highly active regions of the soil such as the rhizosphere. Nevertheless, all members of a particular (cultured) taxonomic unit (e.g., species) have many phenotypic characteristics in common and this can help in relating their presence to their ecosystem function.

In describing the characteristics of prokaryotes, we will necessarily focus on established, cultivated groups, highlighting the ecological relevance of these characteristics and demonstrating importance through species diversity and functional diversity and assessing the extent to which these can be related. Understanding the characteristics of the "significant majority" of other prokaryotes awaits new techniques for their isolation and/or cultivation-independent analysis.

## GENERAL FEATURES OF PROKARYOTES

The majority of prokaryotes are smaller than eukaryotes and cell size per se has significant influence on their ecology, methods of their study, and perceptions of their importance. Prokaryotic cells are in the order of several micrometers in length or diameter, although there are notable exceptions (Schulz and Jorgensen, 2001). The fact that bacteria cannot be seen with the naked eye fools many into believing that they are not important in soil processes and leads to approaches in which prokaryotes are treated as a "black box," with little consideration of their enormous species richness and diversity. Microscopic size also makes observational studies difficult and leads to study of populations or communities, rather than individuals, and to estimating characteristics (e.g., cell concentrations) on the basis of properties of samples.

Small size is associated with high surface area:volume ratio, which explains, in part, the ability of prokaryotes to sequester nutrients at extremely low concentrations. Cells are in intimate contact with their physical and chemical environment. Although homeostatic mechanisms exist for maintaining internal solute concentrations and pH, prokaryotes respond much more rapidly to, and are influenced more by, changes in environmental conditions than the more complex cells of eukaryotes. This, in turn, necessitates greater consideration of microenvironments or microhabitats and the physicochemical characteristics of the environment immediately surrounding the cell. The 1–10 μm scale will be of greater significance for growth and activity of unicellular organisms than for bulk soil properties. Again, this has methodological implications. Small size also influences the distribution and movement of organisms. For example, prokaryotes are able to penetrate and colonize small soil pores, potentially protecting them from predation.

## CELL STRUCTURE

### UNICELLULAR GROWTH FORMS

Cell shape depends on internal turgor pressure acting against cell wall components, whose elasticity varies during cell growth. These interactions can be used to explain the differences between rod-shaped and coccoid cells and some of the more detailed aspects of cell shape and morphology. In addition, there is increasing evidence for the involvement of an actin-like skeleton controlling cell growth and shape in bacterial cells. In fact, unicellular bacteria exhibit a wide range of cell forms, including spiral cells, vibrios, pleomorphic cells, stalked cells, "bacteroids," and even square bacteria. Archaea also exhibit a range of unusual growth forms, although the mechanisms generating and ecological significance of these forms are unclear.

The shape, form, and size of prokaryotic cells are important characteristics when considering the ecology of bacteria in the soil. Nutrient uptake will be determined, to some extent, by the surface area:volume ratio for a cell. This factor has been shown to contribute to the ability of spiral-shaped bacteria (e.g., *Spirillum*) to outcompete rod-shaped pseudomonads under conditions of substrate limitation. Shape and size may also be important in susceptibility to predation, with evidence that protozoa and flagellates choose prey partly on the basis of these factors.

Bacterial shape and size are not fixed and, for *Escherichia coli*, cell volume can vary 27-fold depending on growth rate. In addition, many bacteria take on different forms depending on environmental conditions. A striking example is the $N_2$-fixing rhizobia, which form nodules on the roots of leguminous crops (Chap. 14). Initial contact with the plant root is through attraction to the root of flagellated, motile, rod-shaped bacteria. After infection, flagella are shed and bacteria form swarmer cells. Rapid division of these cells leads to formation of infection threads and then nodules that contain masses of *Rhizobium* cells, the majority of which are misshapen bacteroids, with bulging cell walls and unusual morphologies. Another example is *Arthrobacter*, commonly isolated from the soil, which grows as rod-shaped blue cells at high growth rates and as coccoid purple cells under nutrient-limiting conditions.

These effects are accentuated in starving cells and the transition from growth to starvation is frequently associated with a significant decrease in cell size, changes in cell characteristics (Fig. 5.3), rapid turnover of cell material, a decrease in ribosome number, and expression of a suite of starvation genes (Kjelleberg, 1993). Many of these genes encode high-affinity nutrient uptake systems with broad substrate specificity. These enable cells to sequester a wider range of substrates at low concentrations, giving them an advantage over organisms that, under conditions of nutrient excess, have much greater maximum specific growth rates. Starved forms are resistant to environmental stress and, although not as resistant as bacterial endospores, starved vegetative cells can survive better than growing cells. Direct observation of prokaryotic cells in soil indicates that many are much smaller than typical laboratory-grown organisms, with significant proportions of cells passing through 0.4 μm pore size filters. There is evidence that these cells are less able to grow in laboratory culture.

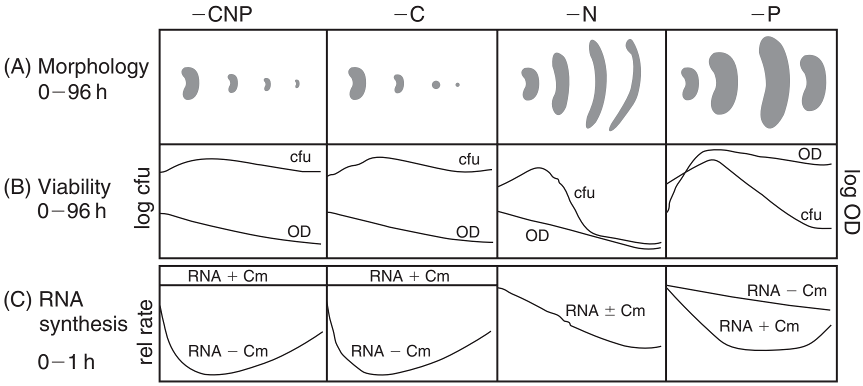



FIGURE 5.3 Changes in (A) morphology, (B) viability, and (C) RNA synthesis (in the presence and absence of chloramphenicol) in *Vibrio* during starvation for combined and individual C, N, and S. The different forms of starvation lead to significant changes in cell size and/or cell morphology and have different effects on changes in RNA content and in biomass and cell concentrations. Chloramphenicol was added to inhibit protein synthesis. Redrawn from Östling *et al.* (1993).

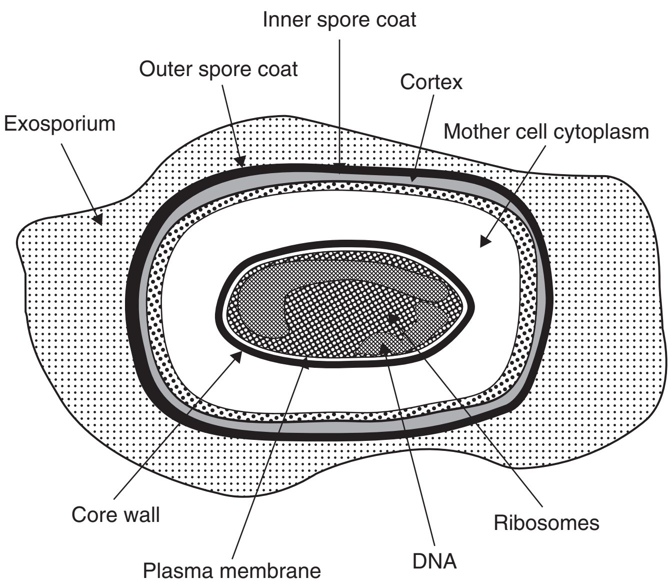



FIGURE 5.4 Major characteristics of a bacterial spore.

A limited number of microbial groups, notably bacilli and clostridia, produce internal spores, termed endospores (Fig. 5.4). These structures are highly resistant to extremes of temperature, radiation, pressure, and other forms of environmental stress. They are the most resistant biological structures known and, although the environmental extremes to which they are resistant are rarely encountered in soil, they are responsible for the persistence and survival of these organisms over many years and even centuries.

## FILAMENTOUS AND MYCELIAL GROWTH

Although prokaryotes are typically considered unicellular, a number of groups exhibit filamentous growth. Some bacteria (streptococci) and cyanobacteria (e.g., *Nostoc*) grow filamentously, not as continuous hyphae, but as chains of cells. In some cases, this is because dividing cells do not separate completely but in others it provides some of the advantages of true mycelial organisms in allowing mycelia to compartmentalize and differentiate. For example, in $N_2$-fixing cyanobacteria, anaerobic conditions required for $N_2$ fixation can be localized in some cells, called heterocysts, while others carry out oxygenic photosynthesis. Chemical communication between cells allows two-way flow of nutrients and also signaling processes leading to regular distribution of $N_2$-fixing cells along filaments.

The actinobacteria (previously termed the actinomycetes) exhibit the greatest variety of growth forms, ranging from single-celled rods and cocci to mycelial structures (Prosser and Tough, 1991). For example, arthrobacters and some rhodococci grow as single cells that do not completely separate and thus do not form true mycelia, but which may subsequently fragment. Others, such as *Nocardia*, are dimorphic and form true, branching hyphal structures during early growth, which then fragment as conditions become less favorable. The hyphal form facilitates colonization of soil particles and, potentially, movement across barren regions to new nutrient sources. Fragments may subsequently develop centers of mycelial growth and their major function may be in dispersal.

The most highly developed mycelial structures are formed by streptomycetes, which grow as branched hyphae forming a true mycelium, similar to those of filamentous fungi (Chap. 6). Hyphal fragmentation may occur under certain conditions, but the major means of dispersal is through exospores borne on aerial hyphae. Exospores do not exhibit the high levels of resistance to environmental extremes of endospores, but are very resistant to desiccation. This enables their survival in the soil, but is also an important factor in their very effective dispersal through the atmosphere. On solid medium, spores develop at the center of actinobacterial colonies fueled by lytic products of substrate mycelium, which stop growing as nutrients become limited. These organisms therefore achieve growth over short distances by extension and branching of hyphae and dispersal by producing high numbers of single-celled spores.

## CELL WALLS

Prokaryotic cells are surrounded by a generally rigid cell wall, protecting the cell from osmotic lysis (Figs. 5.5 and 5.6). The cell wall has also been important in identification and classification of bacteria, providing a major division between gram-positive and gram-negative bacteria, defined on the basis of the Gram stain. The cell wall of gram-positive cells consists of a single thick layer of peptidoglycan, surrounding the cytoplasmic membrane. Peptidoglycan is a polymer consisting of a backbone of alternating *N*-acetylglucosamine and *N*-acetylmuramic acid residues connected to cross-linked peptide chains of four amino acids. Gram-positive cell walls also usually contain teichoic acids, polymers of glycerol or ribitol, linked by

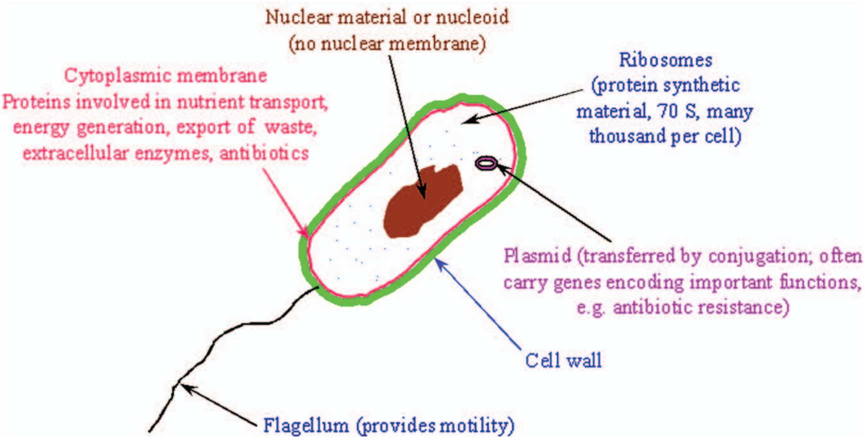



FIGURE 5.5 Illustration of the major characteristics of prokaryote cell structure. (See Fig. 5.6 for detailed structure of the bacterial cell wall.)

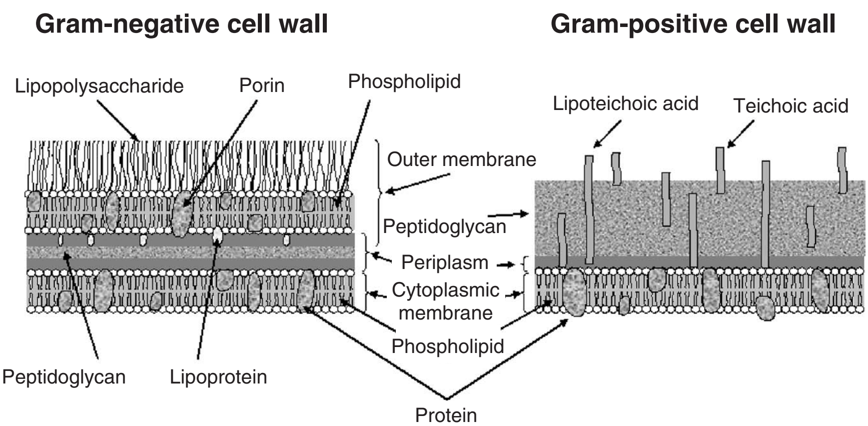



FIGURE 5.6 Detailed structure of gram-negative and gram-positive cell walls.

phosphate groups and containing amino acids and sugars. In the more complex gram-negative cell wall, the peptidoglycan layer is much thinner and is surrounded by an outer membrane enclosing a periplasmic space, which contains enzymes involved in nutrient acquisition, electron transport, and protection from toxins. In contrast, archaeal cell walls have variable chemical structure, consisting of proteins, glycoproteins, or polysaccharides, but do not contain peptidoglycan (Fig. 5.6).

The cell walls of many bacteria are encased within extracellular material (Fig. 5.5), ranging from apparently rigid and distinct capsules of specific thickness to more diffuse (chemically and physically) extracellular polymeric substances. Many roles have been assigned to this material, including protection from predation, adhesion

to solid surfaces, and biofilm formation. In the free-living $N_2$-fixing bacterium *Azotobacter*, extracellular material is important in creating anaerobic regions required for $N_2$ fixation. Biofilm formation is particularly important, with suggestions that the majority of the soil microbial community is attached to particulate matter (clay minerals, soil organic matter, plant roots, and animals). Particulate material provides a concentration of nutrients necessary for microbial growth, and surface attachment has been shown to increase survival of bacteria and to protect them from environmental stress, including low pH, starvation, and inhibition by antibiotics and heavy metals. An example is the production by nitrifying bacteria, in model soil systems, of copious amounts of extracellular material that effectively forms a blanket over colonies, such that individual cells are not visible. This occurs despite the fact that these autotrophic organisms gain barely sufficient energy from oxidation of ammonium or nitrite, use much of this energy to generate reducing equivalents, and require more reducing equivalents because of the requirement to fix $CO_2$. However, once formed, biofilms of these organisms are protected from a wide range of factors to which suspended cells are susceptible. Attachment of cells to surfaces is also facilitated by short, hair-like fimbriae, while similar structures, sex pili, are involved in cell–cell contact associated with plasmid transfer.

## INTERNAL STRUCTURE

Prokaryotes lack internal, membrane-bound organelles but exhibit diverse internal structures and some differentiation (Fig. 5.5). In bacteria, the cytoplasm is enclosed by a cytoplasmic membrane consisting of ester-linked, straight-chained fatty acids. The lipids comprising the membrane form a bilayer, with non-polar hydrophobic ends associating with each other and polar, hydrophilic ends externalized. Cytoplasmic membranes in Archaea contain only a single lipid layer consisting of ether-linked, branched aliphatic acids. For all prokaryotes, the cytoplasmic membrane provides an important link between the cell and its environment and contains many proteins required for import of nutrients, export of waste products, and production of extracellular enzymes for breakdown of high-molecular-weight compounds. It is also involved in generating energy in respiring cells through oxidative phosphorylation and can provide protection through toxin or antibiotic degrading enzymes.

The bacterial chromosome is present as a single, double-stranded, covalently closed, circular DNA molecule forming a nucleoid. Additional genetic material may be present as one or many small DNA molecules, termed plasmids, having a similar structure. These can be transmitted vertically (from generation to generation) and horizontally or laterally, between different bacterial strains. Other mechanisms of genetic exchange in prokaryotes are transformation, which involves direct uptake of DNA and incorporation of genes into the host chromosome, and transduction, in which gene transfer is mediated by a bacteriophage.

Proteins are synthesized within the cytoplasm by thousands of ribosomes, often forming structures termed polysomes and attached to mRNA. Storage products

can also accumulate in cells. Examples include poly-β-hydroxybutyrate (a glucose polymer), polyphosphate, and glycogen, while the sulfur oxidizer *Beggiatoa* stores elemental sulfur, which can be seen as yellow granules within the cell.

Some intracellular structures are associated with specific metabolic processes. Autotrophic bacteria possess carboxysomes, which are particulate bodies involved in fixing carbon dioxide. Photosynthetic bacteria possess complex intracellular membrane structures, which are the site of the energy-trapping, photosynthetic processes. Photosynthetic bacteria show relatively close evolutionary relationships to other functional groups with similar complex membrane structures involved in other reactions, including ammonia, methane, and iron oxidation. Membranes can occur in distinctive patterns, for example, forming a layer within the cytoplasmic membrane or in an equatorial plane, and are diagnostic for some groups.

### MOTILITY

Many prokaryotes are motile, the most obvious mechanism being "swimming" by rotating one (polar), two, or many (peritrichous) flagella. The flagellum consists of a long, helically shaped protein (flagellin) anchored in the cytoplasmic membrane and extending through the cell wall. Rotation of the flagellum leads to movement of the cell. Movement in the soil environment is important for unicellular organisms searching for new sources of nutrients. Flagellar motility is, however, unlikely to be useful for transport between pore size classes in soil as rates of movement are not great. Nevertheless, flagellar motility can be linked to chemotaxis (movement toward a chemical attractant), which appears to be important for communicating between bacterial cells and plant roots in the soil environment, e.g., in movement of rhizobia toward roots prior to nodule formation. Movement over long distances is, however, more likely to be achieved through bulk flow of water containing suspended cells or carriage on roots or soil animals. Indeed, lack of motility is probably important in the formation of microniches occupied by microcolonies of organisms, reducing competitive interactions, reducing selective sweeps, and increasing soil bacterial diversity (see Chap. 11). Other mechanisms exist for bacterial motility. For example, spirochetes possess an axial filament that enables the cell to move by flexing and spinning. Other organisms, including cyanobacteria, *Cytophaga*, and mycobacteria, can move by gliding over surfaces.

## METABOLISM AND PHYSIOLOGY

### CARBON AND ENERGY SOURCES

The phylogenetic classification of prokaryotes described earlier in this chapter provides an indication of evolutionary relationships, which will have arisen, in part, through differences in physiological characteristics. As a consequence, molecular phylogeny often agrees with traditional classification based on combinations of

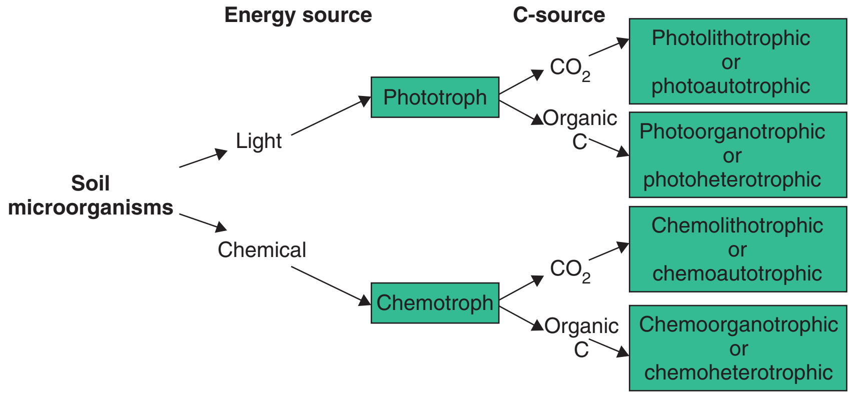



FIGURE 5.7 Physiological classification of soil bacteria in terms of their C and energy source (lithotrophs and organotrophs).

physiologies. Physiological classification is also valuable when considering the role of prokaryotes in soil processes. However, many physiological processes have evolved at different times in different organisms, and particular physiological characteristics may therefore be found in many genera and species. This is illustrated in Tables 5.1 and 5.2 and will be exemplified in greater detail in examples below.

Physiological classification of soil microorganisms is of great value to those interested in ecosystem functions as it provides one of the underpinning bases for considering ecological roles (Fig. 5.7). The first order of such a classification relates to the energy source of the microorganisms. Phototrophs use light and chemotrophs use chemical energy as their energy source. The second order usually relates to the C source. Autotrophs or lithotrophs use $CO_2$, while organotrophs or heterotrophs use organic compounds. Cyanobacteria and green sulfur bacteria are examples of photoautotrophs, while the purple non-sulfur bacteria are photoheterotrophs. S- and Fe-oxidizing thiobacilli and nitrifiers (oxidizing reduced forms of N) are examples of chemoautotrophs, while pseudomonads and *Rhizobium* are examples of chemoheterotrophs.

Although the standard physiological classification based on energy/C source described above enables links to be made to potential ecosystem functions, there are exceptions to the above rules. For example, heterotrophic bacterial nitrifiers have a range of complex physiologies that cannot always be readily classified according to Fig. 5.7, not least because some appear to change their physiological strategy with time and with the availability of energy sources (Killham, 1986). This concept of physiological flexibility will be developed further below.

## OXYGEN REQUIREMENTS

The requirement for the presence or absence of molecular oxygen provides a useful basis for further classifying soil bacteria and a useful indicator of ecological

niche. Obligate aerobes such as *Rhizobium* (a chemoheterotroph), *Thiobacillus* (a chemoautotroph), and many other soil bacteria require oxygen, which acts as a terminal electron acceptor during aerobic respiration. Obligate anaerobes such as *Clostridium pasteurianum* require the absence of molecular oxygen and, instead of using inorganic electron acceptors, remove hydrogen atoms from organic compounds and dissipate them through reaction with products of carbohydrate breakdown. Facultative anaerobes, such as the denitrifying chemoheterotroph *Pseudomonas aeruginosa* or, less commonly, the chemoautotroph *Thiobacillus denitrificans*, can grow in the presence or absence of oxygen. For these denitrifiers, nitrate substitutes for oxygen as an alternative terminal electron acceptor during anaerobic respiration and is reduced to either nitrous oxide or free $N_2$. The switch from aerobic to anaerobic metabolism is quite rapid (occurring within a few hours in most cases) and is controlled by oxygen availability:

The stepwise reduction of nitrate to $N_2$ by facultatively anaerobic denitrifying bacteria occurs as follows:

| | |
|---|---|
| (1) $NO_3^- + H_2 \rightarrow NO_2^- + H_2O$; | (1) nitrate → nitrite; |
| (2) $2NO_2^- + 3H_2 \rightarrow N_2O + 3H_2O$; | (2) nitrite → nitrous oxide; |
| (3) $N_2O + H_2 \rightarrow N_2 + H_2O$ | (3) nitrous oxide → free $N_2$. |

Because of the accumulation of incompletely oxidized products during growth, the energy yields from anaerobic metabolism in soil are generally low (often only a few percent) compared to aerobic metabolism.

It is becoming increasingly clear that while the classification in Fig. 5.7 holds true, many aerobic processes are carried out in soil using sparingly available supplies of oxygen by microaerophiles, obligate aerobes that grow best at low oxygen tensions.

## SUBSTRATE UTILIZATION

The degradative and overall enzyme profile of a soil bacterium determines the range of substrates it can use, although other factors (e.g., substrate availability, competition, environmental factors), considered in subsequent sections, also determine which substrates are being used at any given time. This concept of enzymatic profile applies to both chemoheterotrophs and chemoautotrophs. Table 5.3 highlights just some of the enzymes controlling both intracellular and extracellular use of substrates. The size of many of the substrates (e.g., lignin, cellulose) ensures that most depolymerization is extracellular, with only the final use of the simple building blocks being intracellular. Table 5.3 is by necessity a simplification as, in a number of cases, there is a suite of enzymes, rather than a single enzyme operating on the substrate in question. The enzymes listed in Table 5.3 can be classified as oxidoreductases (e.g., glucose oxidase, ammonia monooxygenase, hydroxylamine oxidoreductase, nitrite oxidoreductase, methane monooxygenase), transferases (e.g., amino transferases), hydrolases (e.g., protease, urease, amylase, ligninase,

TABLE 5.3 Selected Examples of Enzymes and Associated Bacteria Involved in Organic Substrate Utilization

| Degradative enzymes used for organic substrate utilization | Distribution of enzymes/examples of soil bacteria with ecologically significant activity of these enzymes |
|---|---|
| Cellulase (cellulose → glucose subunits) | Species of *Bacillus*, *Cellulomonas*, and *Pseudomonas* |
| Glucose oxidase (glucose → $CO_2$) | Ubiquitous enzyme among soil bacteria |
| Protease (protein → amino acids) | Widespread among soil prokaryotes but species of *Pseudomonas* and *Flavobacterium* are strongly proteolytic |
| Deaminase/amino transferase; amino acid decarboxylase (removal of amino and carboxyl groups to liberate $NH_3$ and $CO_2$ from amino acids) | More common enzymes than proteases, although major differences in rates between amino acids |
| Urease (urea → ammonia + carbon dioxide) | About 50% of heterotrophic soil bacteria are ureolytic |
| Amylase and glucosidase (starch → glucose) | Species of *Bacillus, Pseudomonas*, and *Chromobacterium* |
| Ligninase (lignin → aromatic subunits) | While lignin degradation is primarily the domain of the white rot fungi, species of *Arthrobacter*, *Flavobacterium*, and *Pseudomonas* are sometimes involved |
| Pectinase (pectin → galacturonic acid subunits) | Species of *Arthrobacter*, *Pseudomonas*, and *Bacillus* (some species possess all of the pectinase enzymes—polygalacturonase, pectate lyase, pectin lyase, and pectin esterase); many plant pathogens possess pectinase to assist in plant host penetration |
| Phosphatase (phosphate esters → phosphate) | About 30% of heterotrophic soil bacteria possess phosphatase enzymes |
| Sulfatase (sulfate esters → sulfate) | Many fewer possess sulfatase |
| Invertase (sucrose → fructose + glucose) | Particularly active in saprotrophic soil bacteria, such as species of *Acinetobacter*, and *Bacillus* |
| Chitinase (chitin → amino sugar subunits) | The actinobacteria *Streptomyces* and *Nocardia* |
| Amino acid decarboxylase | Both aromatic and nonaromatic amino acid decarboxylases are found in a wide range of soil bacteria synthesizing amino acids |

pectinase, phosphatase, sulfatase, invertase, chitinase), and lyases (e.g., amino acid decarboxylase).

Some of the enzymes highlighted in Table 5.4 are constitutive, while others are inducible. A more comprehensive yet highly accessible account of soil enzymes can be found in the review by Burns and Dick (2002).

TABLE 5.4 Selected Examples of Enzymes, Functional Genes, and Associated Soil Bacteria Involved in Autotrophic Oxidation of Substrates

| | |
|---|---|
| Ammonia monooxygenase, *amo*A (ammonia → hydroxylamine) | *Nitrosomonas*, *Nitrosospira*, *Nitrosococcus* |
| Hydroxylamine oxidoreductase, *nor*B (hydroxylamine → nitrite) | *Nitrosomonas*, *Nitrosospira, Nitrosococcus* |
| Nitrite oxidoreductase (nitrite → nitrate) | *Nitrobacter*, *Nitrospira* |
| Methane monooxygenase, *pmo*A | Methanotrophs |
| | Methylotrophs such as *Methylomonas* and *Methylococcus* |

**AUTOCHTHONY AND ZYMOGENY**

Identifying a soil bacterium as a chemoheterotroph, according to the classification scheme for metabolism outlined above, provides little information on how competitive that particular bacterium will be under particular conditions of substrate supply. The great 19th century soil microbiologist Winogradsky addressed this issue through reference to the comparative kinetics of growth, which relates substrate concentration to specific growth rate. In Fig. 5.8, which illustrates some simple and contrasting growth curves, population X (diamonds) will outcompete populations Y and Z at high substrate concentrations. At intermediate substrate concentrations, population Y (circles) will outcompete populations X and Z. At low substrate concentrations, however, population Z (triangles) will outcompete the other populations. Soil bacteria that exhibit the growth kinetics of population X, with a relatively high maximum specific growth rate ($\mu_{max}$) and substrate affinity ($K_s$), will be more competitive at high substrate concentrations and are termed "zymogenous." Not surprisingly, soil microenvironments such as the early rhizosphere are dominated by zymogenous bacteria, such as fluorescent pseudomonads, which grow rapidly on the simple C substrates (primarily glucose). Soil bacteria that exhibit the growth kinetics of population Z, with a low $\mu_{max}$ but relatively low $K_s$ (i.e., a high substrate affinity), are termed "autochthonous." The bacterial populations found in some of the less accessible soil microenvironments (e.g., the smaller pores inside soil aggregates), where substrate C flow is rarely more than a trickle, are generally dominated by autochthonous bacteria. The spatial variability of soil with regard to microbial populations highlights the importance of substrate (and nutrient) availability (sometimes referred to as bioaccessibility) as a driver of both the diversity and the function of the bacterial community. The latter can be demonstrated by studying the mineralization of differentially located (in terms of soil pore size class), radiolabeled C substrates (Killham *et al.*, 1993).

Of course, in reality, there are degrees of autochthony and zymogeny, as indicated by the growth kinetics of the populations in Fig. 5.8. There is a continuum of growth kinetics that ensures that the most competitive soil bacteria will change with substrate concentration. Successions will therefore often occur in environments

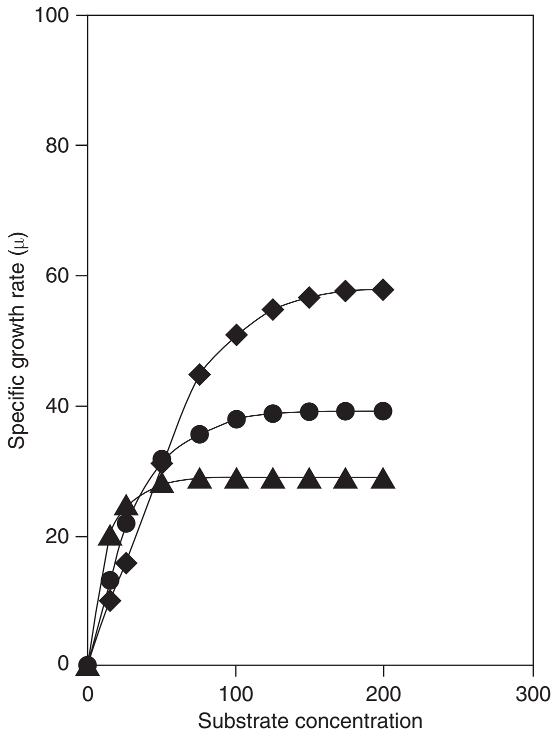



FIGURE 5.8 Comparative Monod kinetics used to assess substrate competition for growth between soil microbial populations.

such as the rhizosphere, where C flow changes, although the picture is further complicated by the proliferation of substrates with varying recalcitrance, enzyme specificity, and availability.

## OLIGOTROPHY, COPIOTROPHY, AND THE R–K CONTINUUM

The physiologically based autochthony–zymogeny classification pioneered by Winogradsky is often considered analogous to the r–K continuum commonly used in plant and animal ecology, based on logistic models, and to the scheme of oligotrophy–copiotrophy. The K strategists and oligotrophic bacteria are adapted to growth under conditions of C/nutrient starvation ("oligocarbotrophy" specifies C starvation, while the terms "oligonitrotrophy," "oligophosphotrophy," etc., specify the type of nutrient starvation). Copiotrophs are adapted to nutrient excess. Although the two schemes are used in similar ways, the contrast in physiological versus logistic approaches does distinguish them. Furthermore, oligotrophy is also considered to include unusual forms of C (and nutrient) scavenging, such as

exploiting gaseous C sources such as $CO_2$ (e.g., through anapleurotic $CO_2$ fixation) and volatile organic acids.

### FACULTATIVENESS

While formal functional/physiological classification of bacteria is a useful foundation, it does not embrace the flexibility exhibited by many organisms. For example, we know that many soil bacteria that can grow readily on "standard" organic substrates can adapt and continue to metabolize under the harsh conditions of C starvation often found in soil. The physiological strategies and mechanisms used by the "facultative oligotrophs" are only partially characterized and include scavenging of gaseous forms of C, which diffuse through the soil pore network.

Perhaps the best known example of facultativeness relates to oxygen requirements. Facultative anaerobes metabolize most efficiently as aerobes. Indeed the denitrifying pseudomonads, which often colonize the rhizosphere as aerobic chemoheterotrophs, may seldom experience low oxygen concentrations that induce the nitrate reductase enzyme system associated with denitrification. Many microaerophiles also often operate under well-aerated conditions but, as facultative microaerophiles, are physiologically adapted to life at the low oxygen concentrations sometimes experienced in wet soils, particularly in microsites such as water-saturated aggregates beyond a critical radius that prevents adequate diffusive resupply after removal by respiratory (roots, animals, and microorganisms) demand (Greenwood, 1975).

Another aspect of flexibility is the capacity to metabolize more than one substrate, often simultaneously (for example, through cometabolism, see Chaps. 9 and 17), which may be the norm rather than the exception. This is reflected in the diversity of catabolic enzymes, both intracellular and extracellular. Among the most versatile degraders in the soil are the pseudomonads. They can degrade the most complex aromatic structures through to the simplest sugars (Powlowsky and Shingler, 1994). *Pseudomonas cepacia*, for example, can metabolize more than 100 different C substrates (Palleroni, 1984).

Flexibility is also evident from the way soil microbial communities have adapted rapidly to degrade new substrates that have been introduced as contaminants in soil, such as organoxenobiotics that have been synthesized *de novo* by industry. The development of populations with appropriate catabolic genes is one of the greatest phenomena exhibited by the soil microbial community and is discussed further below.

## BIODEGRADATION CAPACITY

### CELLULOSE

Plant residues provide the major source of soil organic matter and their biodegradation is critical to ecosystem productivity. Because plants typically contain up to

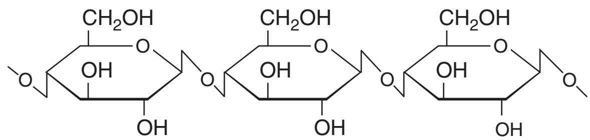



FIGURE 5.9 The chemical structure of cellulose, which consists of β-1,4-linked glucose molecules.

60% cellulose (Paul and Clark, 1989), the decomposition of cellulose is a key activity of soil bacteria and it is vital to the energy flow through soils and to the cycling of N, P, and S (the decomposition of cellulose is generally accompanied by immobilization of these nutrient elements).

In simple terms, the decomposition of cellulose is a relatively specialized depolymerization exercise (involving a restricted number of saprophytes) followed by hydrolysis to the simple sugar glucose, which is rapidly used as an energy source by most heterotrophic soil microorganisms. The cellulose polymer occurs in plant residues in a semicrystalline state and consists of glucose units joined by β-1,4 linkages, with chains held together by hydrogen bonding (Fig. 5.9) (see Chap. 12 for further details).

The cellulase enzyme complex, which catalyzes cellulose decomposition, occurs in a large number of cellulolytic bacteria (e.g., species of *Bacillus*, *Pseudomonas*, *Streptomyces*, and *Clostridium*) and fungi and operates a two-stage process. The first involves "conditioning" by decrystalizing cellulose and the second involves extracellular depolymerization units, eventually forming double to single sugar units by the enzyme cellobiase. Although the half-lives and turnover times of cellulose and hemicellulose in soil are on the order of days and weeks, glucose metabolism after cellulose depolymerization is extremely rapid (in the order of hours to a day) (Killham, 1994).

The term hemicellulose describes various sugar (hexoses and pentoses) and uronic acid polymers that, like cellulose, are decomposed by a relatively specialized depolymerization process, followed by a much more rapid assimilation and oxidation of the simple monomer. Pectin, a polymer of galacturonic acid subunits, provides a good example of this, with specialist pectinolytic bacteria such as species of *Arthrobacter* and *Streptomyces* producing the extracellular pectin depolymerases (exo- and endo-) and then a much wider range of heterotrophic soil microorganisms using galacturonic acid oxidase to exploit the energy bound in the subunit itself (Killham, 1994).

## POLLUTANTS

With continually expanding industry and a global dependence on fossil fuel hydrocarbons as well as agrochemicals, there are few environments that are not in some way affected by a spectrum of organic pollutants. These organic pollutants

include the aliphatic hydrocarbons (e.g., alkanes from oil spills and petrochemical industry activities), alicyclic hydrocarbons (e.g., the terpenoid plant products), aromatic hydrocarbons (e.g., single aromatics, such as the petrochemical solvents benzene and toluene, and polyaromatics, such as pyrene), the chlorinated hydrocarbons (e.g., chlorinated aliphatics such as chloroform), chlorinated aromatics (e.g., chlorobenzenes and chlorinated polyaromatics such as PCBs, DDT, and dioxins), and N-containing aromatics (e.g., TNT). Because of the increasing awareness of the adverse effects of these organoxenobiotic pollutants, the soil microorganisms and associated genes (largely carried on plasmids, which are extrachromosomal pieces of DNA) involved in their degradation are of great interest to soil microbiologists. This interest focuses on the pollutant biodegradation capacity of microorganisms (which refers to both the breadth of the pollutants that they degrade and the degradation rates) and also on bioremediation of contaminated environments (Crawford and Crawford, 1996).

Some of the organic pollutants entering the soil environment are toxic to bacteria and are recalcitrant to mineralization. The reasons for the latter are numerous and include key physicochemical characteristics such as the way in which the pollutant partitions into the soil matrix—relevant only if related to metabolic processes. For example, the more hydrophobic polyaromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) tend to bind strongly to the soil solid phase and limit microbial/enzyme access. Another reason for recalcitrance is the large number of enzyme-regulated steps and the number of well-regulated genes required for mineralization. Furthermore, since many of the organic pollutants under consideration are synthetic, there are often no corresponding genes coding for proteins existing in extant soil bacteria that can immediately catalyze the degradation process.

The highly chlorinated organic pollutants have been studied extensively in terms of their degradation and the evolution of their degradative genes. Although degradation is generally slow, particularly for the more chlorinated compounds, gene clusters have clearly evolved for their degradation. Structural and regulatory genes have high sequence homology, even though they have evolved as plasmid-borne genes in different soil bacterial genera from across the world (Daubaras and Chakrabarty, 1992). This evolution of gene-regulated bacterial degradative pathways (usually involving four to eight genes in each case) enables complete mineralization of a range of chlorinated pollutants, including chlorobenzenes used in a number of industrial processes and the chlorophenoxyacetic acids associated with some of the hormonal herbicides used in agricultural weed control. The degradation of many of the chlorinated organic pollutants is somewhat complicated by the fact that the bacterial removal of chlorine from the molecule (dehalogenation) occurs most readily under anaerobic conditions, and so bioremediation of contaminated environments containing both chlorinated and nonchlorinated hydrocarbons can be a complicated process involving management of both anaerobic and aerobic conditions. Although the anaerobic dehalogenation of PCBs is relatively well understood, obtaining reliable and useful bacterial isolates is fraught with difficulties (May *et al.*, 1992).

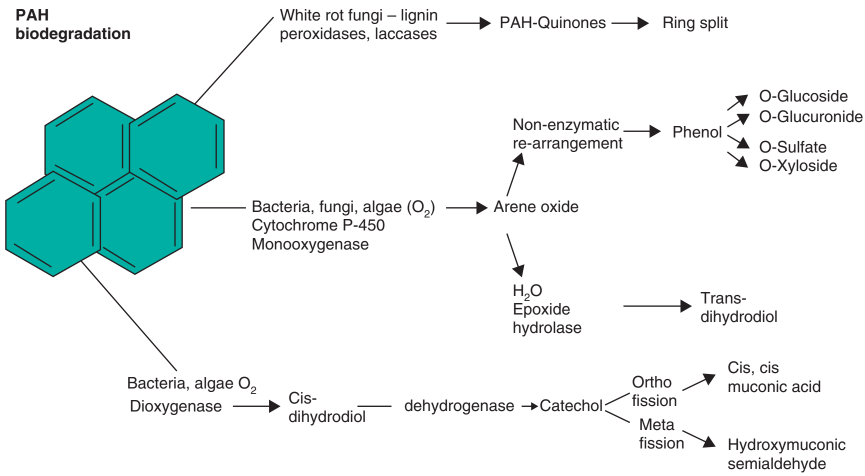



FIGURE 5.10 Schematic of soil microbial metabolism of polyaromatic hydrocarbons.

The soil bacteria and associated genes involved in the degradation of PAHs have, like the PCBs, been extensively studied and exploited in both bioremediation and generating bacterial biosensors for detecting these complex organic pollutants. For the bacteria that aerobically degrade PAHs (e.g., naphthalene, acenaphthylene, fluorene, anthracene, phenanthrene, pyrene, benz(a)anthracene, benzo(a)pyrene), attack often involves oxidation of the rings by dioxygenases to form *cis*-dihydrodiols (Fig. 5.10). The bacteria involved in these oxidative reactions include species of *Mycobacterium* (Kelley *et al.*, 1993) and *Pseudomonas* (Selifonov *et al.*, 1993). The dihydrodiols are transformed further to diphenols, which are then cleaved by other dioxygenases. In many bacteria, this precedes conversion to salicylate and catechol (Sutherland *et al.*, 1995). The latter is a rather toxic, relatively mobile intermediate and any bioremediation of PAHs must consider and manage issues such as this both because of possible environmental hazards and because the catechol may inhibit the bacteria being harnessed for the bioremediation itself!

## DIFFERENTIATION, SECONDARY METABOLISM, AND ANTIBIOTIC PRODUCTION

Differentiation is when there is a change in bacterial activities from those associated with vegetative growth. This phenomenon is particularly associated with cell starvation, during which intracellular components are often transported and resynthesized into new compounds through secondary (i.e., non-growth-linked) metabolism. The compounds formed can include antibiotics, pigments, and even agents such as melanin that are protective against enzymatic attack. Antibiotics are produced by the actinobacteria (e.g., *Streptomyces* and *Actinomyces*) and by *Bacillus* and *Pseudomonas*. Antibiotics are often powerful inhibitors of growth

and metabolism of groups of other microorganisms, with varying degrees of specificity. Streptomycin, for example, produced by certain species of *Streptomyces*, strongly inhibits a wide range of both gram-positive and gram-negative bacteria. Cycloheximide, on the other hand, inhibits only eukaryotes and is used as a fungal inhibitor in the bacterial plate count.

Antibiotic production by soil bacteria, involving secondary metabolism, has been harnessed for decades for a wide range of medical applications. Although antibiotic production has long been linked with chemical defense, the factors determining antibiotic production in soil suggest that antibiosis occurs only when the supply of available carbon is high (Thomashow and Weller, 1991). These conditions are likely to be met in the rhizosphere, the zone around seeds (the spermosphere) and relatively fresh plant or animal residues.

Although a considerable number of antibiotic-producing bacteria have been identified, evidence that antibiosis is a significant chemical defense strategy in the rhizosphere tends to be indirect. For example, strains of fluorescent pseudomonads can demonstrate high specific rates of production of the antibiotic phenazine, which is strongly inhibitory against *Gaeumannomyces graminis* var *tritici* (Ggt), the causal agent of take-all in wheat (Brisbane and Rovira, 1988). When Tn5 mutants of such pseudomonads are introduced into the wheat rhizosphere, the removal of antibiotic production has been associated with reduced control of Ggt (Thomashow and Weller, 1991).

The key genes and associated enzymes involved in antibiotic synthesis have now been characterized in some cases (e.g., for phenazine, see Blankenfeldt *et al.* (2004)) and hence offer new and more powerful approaches to investigate these secondary metabolic, chemical defense strategies of bacteria in soil; by probing for RNA-based antibiotic biosynthesis genes and/or by use of stable isotope ($^{13}C$) probing of the rhizobacterial nucleic acid pool, questions of both the activation and the significance of antibiotic-mediated chemical defense can now be resolved.

The final two sections of this chapter have considered xenobiotics in two forms—antibiotics and persistent organic pollutants such as PCBs and PAHs. Nakatsu *et al.* (1991) pointed out that these two forms of organoxenobiotics have a key role in presenting soil bacteria with a major selection pressure. The molecular bases for organoxenobiotic resistance and catabolism are generally located in soil bacteria, on extrachromosomal plasmid DNA, the maintenance of which requires this selection pressure.

## CONCLUSIONS

The structure and physiology of prokaryotes have been studied for more than a century, and we know much about their taxonomic and metabolic diversity that is directly relevant to their central role and essential activities in soil ecosystems. While summarizing what we know, we have also indicated the vast amount of important information that remains to be discovered. Arguably the majority of phylogenetic

groups and strains that are abundant and important for soil processes have yet to be isolated and characterized. The diversity of the prokaryotes is significantly greater than that of higher organisms, but the lack of clear species definition limits our ability to apply concepts linking, for example, diversity to important ecosystem properties, such as stability and resilience. Elucidation of mechanisms generating and controlling prokaryote diversity within the soil environment and understanding the two-way relationships between prokaryotes and the soil, with its spatial and temporal heterogeneity, represent an enormous and exciting challenge. It is essential that this challenge is undertaken in order to provide a basis for understanding, and potentially, predicting the impact of environmental change on prokaryote diversity. Increased understanding of the links between phylogenetic diversity and functional and physiological diversity is also essential to determine the consequences of changes in prokaryote diversity and community structure on terrestrial biogeochemical cycling processes.

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# 6

# Fungi and Eukaryotic Algae

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## INTRODUCTION

Fungi bind soil together, both literally and figuratively, by their filamentous form, their exudates, and their trophic interactions with all other groups of soil organisms. Soil is fundamentally an aquatic habitat, since water films and water-filled soil pores occur, at least ephemerally, even in dry deserts. For this reason, the eukaryotic algae will be treated together with fungi and fungus-like organisms in this chapter (Fig. 6.1); in addition, both groups share a range of morphologies from filamentous to cellular. Colonial algae and seaweeds with differentiated multicellular organs do not occur in soil and are beyond the scope of this chapter. Cyanobacteria (formerly known as blue-green algae) are prokaryotic members of the Domain Bacteria (=Eubacteria). To treat cyanobacteria as "algae" is as inaccurate and misleading as treating actinomycetes (prokaryotic, GC-rich members of the gram-positive Bacteria) as fungi. The distinction is of particular importance in soil ecology, since many cyanobacteria and some actinomycetes are important in fixation of nitrogen, a capacity not found in any eukaryotes. Both groups are discussed in Chap. 5. The chloroplasts of plants and other photosynthetic eukaryotes have their origins in Cyanobacteria, originally engulfed by an ancestor of the plant lineage (see Fig. 6.1), then transferred by secondary endosymbiosis of an ancestral red alga into the heterokont and alveolate lineage (brown algae through dinoflagellates in Fig. 6.1) or by secondary endosymbiosis of an ancestral green alga into the excavate (euglenoids)

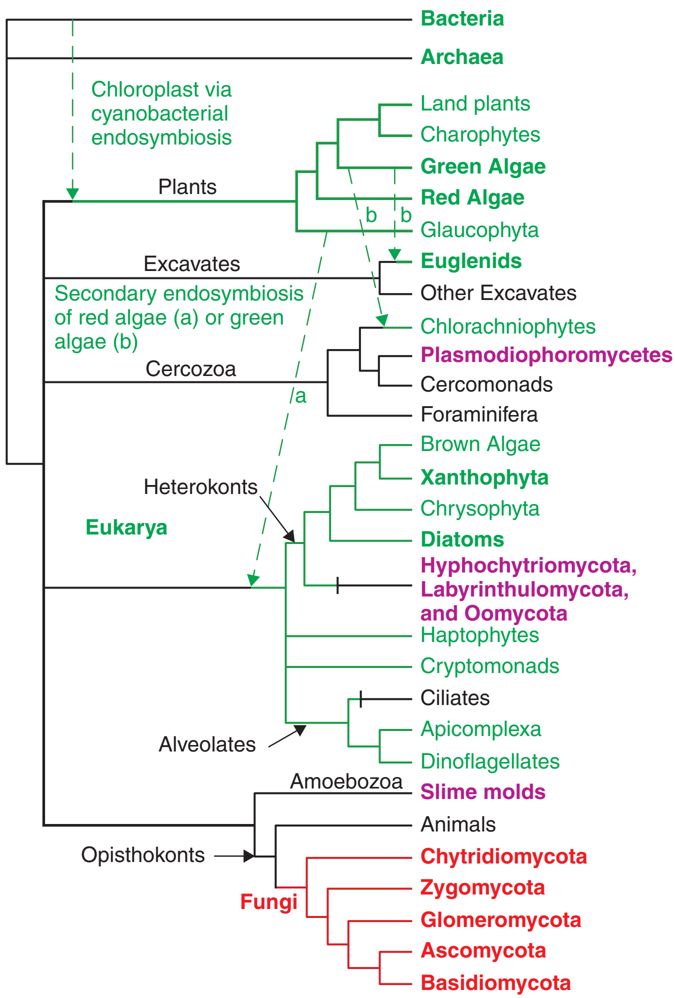



FIGURE 6.1 Possible phylogenetic relationships of major groups of organisms including those covered in this chapter (in bold). Groups with at least some photosynthetic members are indicated in green, and the vertical bars on lines leading to Oomycota and ciliates indicate the secondary loss of chloroplasts that must have occurred if a single endosymbiotic event and monophyly of the group encompassing brown algae through dinoflagellates are accepted. True fungi (Kingdom Fungi) are indicated in red and fungus-like organisms, often studied by mycologists, are indicated in pink. Branch lengths are arbitrary, and a number of polytomies and paraphyletic groups are omitted by simplification. Based on Palmer *et al.* (2004) and Lutzoni *et al.* (2004); for an alternate but largely congruent view of eukaryote relationships, see Baldauf (2003).

and cercozoan (chlorachniophytes) lineages (Palmer *et al.*, 2004). Throughout the tree of life, a number of members of originally photoautotrophic lineages have lost their chloroplasts and become heterotrophic saprotrophs or parasites (notably the Plasmodiophoromycetes, Hyphochytriomycota, Labyrinthulomycota, Oomycota,

and Apicomplexa such as *Plasmodium*, causing malaria). Various features of their ultrastructure and physiology, including sensitivity to selective antibiotics, reflect their true origins.

Fungi are enormously important in C and N cycling because of their ability to degrade complex substrates of plant origin which represent up to 90% of net primary productivity in most terrestrial ecosystems. In addition, the usually mutualistic symbioses known as mycorrhizas between many fungi and plant roots, as well as the parasitic interactions leading to many plant diseases, have huge impacts in both ecological and economic terms (Chap. 10). The true fungi (Kingdom Fungi) are a monophyletic group; that is, the Phyla Chytridiomycota, Zygomycota, Glomeromycota, Ascomycota, and Basidiomycota include all of the known descendents of a single common ancestor, which in turn is closely related to the common ancestor of metazoan animals (Kingdom Animalia; see Chap. 7). Yes, mushrooms and other fungi are among our closest relatives in the tree of life! A number of unrelated groups of eukaryotes that reproduce by spores and lack chlorophyll have traditionally been classified as fungi. These include the Plasmodiophoromycetes (Cercozoa), Hyphochytriomycota, Labyrinthulomycota and Oomycota (Heterokonts), and slime molds (Amoebozoa). Representatives of all of these "aquatic" groups can be found in soil, where some cause major plant diseases and others are detritivores with importance in nutrient cycling. These groups will be treated briefly in this chapter along with the true fungi. Excellent reviews of the ecology of fungi in soil (and other habitats) are presented by Dix and Webster (1995) and Dighton *et al.* (2005). For good general references to fungi, see Alexopoulos *et al.* (1996), Kirk *et al.* (2001), and Mueller *et al.* (2004); for soil fungi, see Domsch *et al.* (1993). Key references to the major groups are listed below where they are discussed.

Their life form and their strengths in biosynthesis and biocatalysis are among the characteristics that make fungi so special. The tubular cell called a hypha (plural hyphae) characterizes most fungi. Hyphae exhibit polar growth. The hyphal tips are expansible and flexible and are where most growth, secretion, and absorption take place. In contrast, the cylindrical side walls are much less metabolically active and become increasingly rigid with age. In theory, a fungal colony that is growing radially increases in biomass exponentially, but under less than ideal conditions (which can even be seen in cultures growing on nutrient-rich medium) the active cytoplasm can be seen to move forward with the expanding hyphal tips, leaving the older parts of the mycelium as empty tubes. This is a remarkable adaptation, which allows a mycelial fungus to explore its environment for food or other resources at a minimal cost. Nutrients in the walls of abandoned hyphae may even be recycled by autolysis and reused in the growing mycelial front. This latter feature is why direct microscopy or measures of *N*-acetylglucosamine digested from the chitinous walls of fungal hyphae (see Chap. 3) often overestimate fungal biovolume relative to measures of fungal DNA (see Chap. 4). Hyphae allow fungi to penetrate solids, whether soil, small particles of decomposing plant litter, a living plant cell, or the wood of a freshly fallen tree, and to traverse the dry spaces between moist or

nutrient-rich microhabitats in soil. This ability leaves bacteria, the other main group of decomposers, behind on the surfaces and gives fungi a huge advantage in decomposition of plant litter and exploration of soil. Numbers of bacteria are primarily regulated by predation by bacterivorous nematodes (and perhaps even bacterivorous fungi; Thorn, 2002), whereas fungi are regulated more by substrate quantity and quality (Wardle, 2002).

The other ability that allows many fungi to greatly outperform bacteria in decomposition of plant litter is their production of lignocellulose-degrading enzymes such as laccases, lignin, and manganese-dependent peroxidases and cellulases. Although cellulases are widespread (if not common) in bacteria, few bacteria (notably certain Streptomycetes) produce lignin peroxidases or have any ability to degrade lignocellulosic plant wastes. In defending their nutritional substrates from other fungi or bacteria, many fungi have developed potent antimicrobial compounds. The fungi are extremely rich sources of novel chemical compounds, often secreted into their environment (including appropriate culture media) as "extralites." Some of these we now exploit in medicine (e.g., penicillins from *Penicillium*, cyclosporin from *Tolypocladium*), whereas others are potent mycotoxins that we do our best to avoid in our food or animal feed (e.g., aflatoxins from *Aspergillus*, fumonisins from *Fusarium*). Mold fungi (anamorphic or asexual Ascomycota) have particularly been exploited for extralites of commercial or medical value, whereas the Zygomycota and Chytridiomycota appear to have little promise in this area, and the Basidiomycota are largely unexplored.

Hyphae of the Glomeromycota and Zygomycota are broad (frequently 10–20 μm in diameter) and coenocytic (with multiple nuclei per cell). Hyphae of the Ascomycota and Basidiomycota are typically much narrower (2–5 μm in diameter, although exceptionally less than 1 or greater than 10 μm in diameter). In most Ascomycota and Basidiomycota, "primary mycelia," formed following germination of a haploid sexual spore, are uninucleate and haploid. "Secondary mycelia," formed following mating of compatible primary mycelia, are binucleate (dikaryotic or heterokaryotic, with two, usually different, haploid nuclei). Union of the two haploid nuclei occurs only in special cells of sexual fruiting bodies (asci in ascomata of Ascomycota or basidia of basidiomata of Basidiomycota) and is immediately followed by meiosis and the formation of haploid sexual spores (ascospores or basidiospores) that repeat the cycle. Several hundreds (or perhaps thousands) of species of Ascomycota and Basidiomycota have evolved a yeast-like growth habit of approximately elliptical cells that reproduce by budding or fission. The ability to grow as a yeast confers an advantage to these fungi when growing in aquatic systems (such as the nectar in flowers, insect hemolymph, or the fermentation of some of our favorite beverages), including some of high osmotic potential. However, the ability to convert back to a hyphal growth form enables certain yeasts (notably *Candida albicans* and its kin) to cause more invasive human infections. Thus, the genetics and biochemistry of yeast–hyphal dimorphism has attracted a great deal of research.

In fungi that form hyphae, the network of these cells that composes the individual is called a mycelium. The fungal mycelium is a powerful ecological force.

Through their mycelium, fungal individuals, species, and communities can dominate three-dimensional real estate (including the O horizon of many forest soils), transfer nutrients across macroscopic distances (meters, instead of the micrometer scale of the individual hypha), interconnect organisms at differing trophic levels, persist through time, and of course marshal the necessary resources to form sexual fruiting bodies. Different fungi have mycelia of considerably different scales. The complete individual of a *Penicillium* might be tens of micrometers in diameter (and produce on the order of $10^6$ spores), whereas the mycelium of many mushrooms (Homobasidiomycetes) may be centimeters or meters in extent (but produce few to no propagules in soil), and the current record holder, a species of *Armillaria*, is known to occupy 40 hectares of forest soil. This has tremendous implications both for their biology and for analysis of their biodiversity, in which replicate "individuals" sampled may represent colonies grown from spores of a single parent mycelium of *Penicillium* or fruiting bodies all formed by a single mushroom mycelium.

It has been estimated that the fungal biomass in many soils exceeds the biomass of all other soil organisms combined, excepting plant roots. This situation is usually found wherever abiotic conditions (low nutrients, periodic or permanent drought, low temperatures, or short growing season) or low-quality litter (high C:N ratio, high lignin:N ratio, or high phenolic content) reduce the rate of litter turnover and nutrient cycling. In certain tropical or agronomic ecosystems, where there are fewer limitations on plant litter decomposition, soil fungi may be much less predominant in terms of their biomass, activities, or diversity. Even in these circumstances, it would be inaccurate to regard fungi as unimportant in the nutrient cycling or other ecological processes, since many parts of these processes take place even before plant litter hits the ground. Certain endophytic fungi, including members of the Xylariales (Ascomycota), switch from relatively quiescent symbionts to saprotrophs as leaves senesce. Dead leaves and other aerial fine litter in the tropics are also quickly colonized by decomposer basidiomycetes such as *Marasmius*, *Mycena*, and *Coprinopsis*—mushroom fungi with small, ephemeral fruiting bodies. Species of *Marasmius* even form a tangled net of tough, melanized rhizomorphs (differentiated hyphal cords) that trap falling leaves in the canopy or subcanopy.

Surveys of the diversity of soil fungi, which were popular during the 1960s and 1970s, have reappeared in the literature with the advent of DNA-based, culture-independent methods of analysis. Culture-based estimates of soil fungal diversity require considerable effort and taxonomic expertise (Chap. 3; see Bills and Polishook, 1994; Maggi *et al.*, 2006). From a single soil sample, several hundred species may be obtained, and new species continue to be encountered after more than 10,000 isolates have been examined (Christensen, 1981)! These estimates, of course, preferentially detect fungi that produce numerous propagules in soil and grow readily on the isolation medium; Basidiomycota are largely overlooked (Warcup, 1950; Thorn *et al.*, 1996). Knowing in advance that there is a high density of fungal species (number of different species per cubic centimeter, or gram dry weight, of soil) warns investigators using DNA-based methods that a large sample size

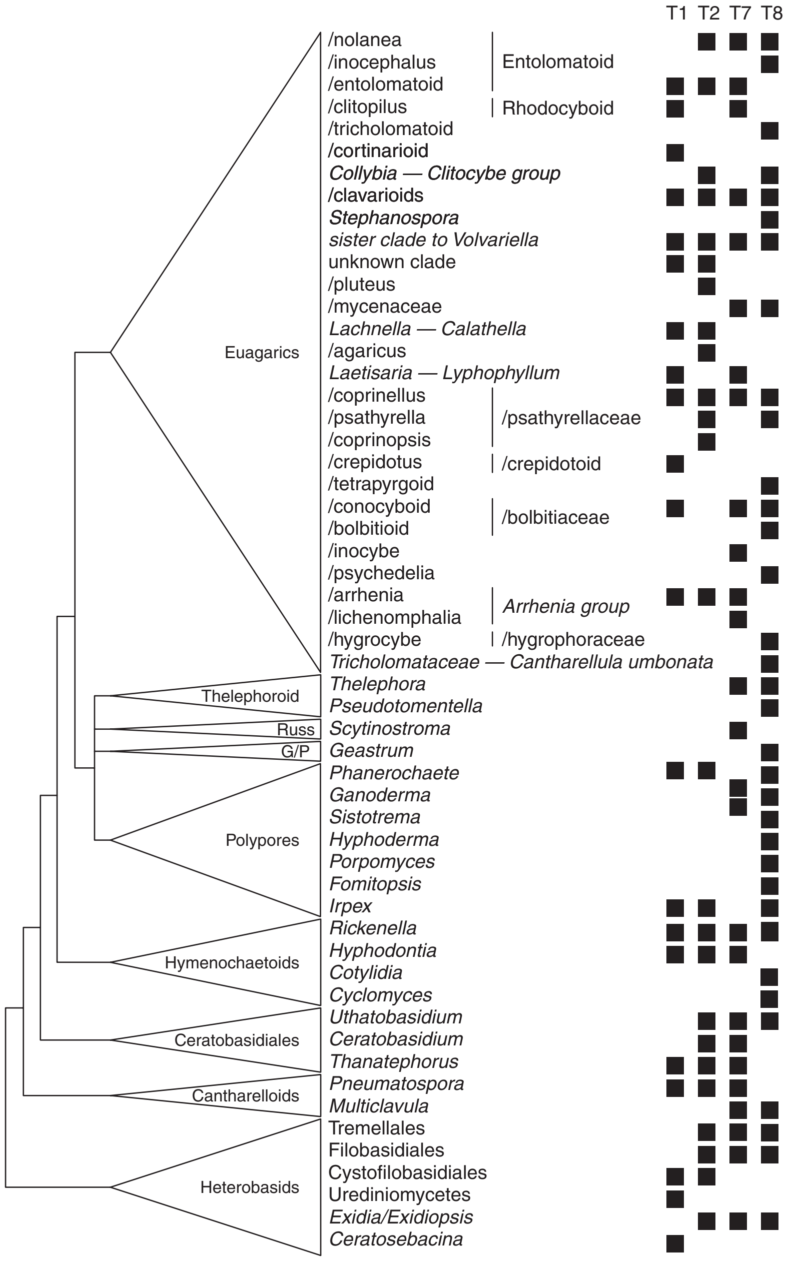



FIGURE 6.2 Phylogenetic relationships of Basidiomycota detected by PCR amplification, cloning, and sequencing of 95 10-g samples from Michigan agricultural soils (Lynch and Thorn, 2006, and unpublished data). The tree was formed by neighbor-joining analysis in PAUP* 4.0b (Swofford, 2002) of all environmental sequences together with all nonredundant reference sequences from GenBank, so that unknown sequences could be named by being placed in terminal clades together with

(number of clones analyzed) will be required to provide a meaningful estimate of the composition and diversity of the community. Even restricting our analyses to soil Basidiomycota, we have found as many as 9 genetic species (where these are defined as having less than 99% similarity over the 5′ 650 bases of nuclear large subunit ribosomal DNA) among 12 clones taken from a single PCR of DNA from a 10-g sample of Michigan agricultural soil (Fig. 6.2; for more methods, see Chap. 4). A total of 241 genetic species were detected in 95 such samples from an 11.2-ha site, and the estimated total species richness for the site is 367, as many as all of the species of macromycetes (larger mushrooms) known from The Netherlands! Some taxa were found across a wide range of agronomic treatments, from corn–soybean–wheat rotation with regular tillage and inputs (T1) to never-tilled native meadow (T8), but the greatest diversity was found in the latter. Basidiomycete diversities in forested ecosystems are predicted to be much higher. So, whether in numbers, biomass, or ecological or biochemical activities, the fungi are important in most soils.

Eukaryotic algae are a polyphyletic group of organisms, derived from several separate lineages: plants (green and red algae and glaucophytes), excavates (euglenoids), cercozoans (chlorachniophytes), and the heterokonts and alveolates (brown algae, xanthophytes, chrysophytes, diatoms, haptophytes, cyryptomonads, apicomlexa, and dinoflagellates). Among these, a number of unicellular, colonial, and filamentous taxa represent an often overlooked component of the soil community. These algae may be locally very important in carbon fixation, soil binding, and nutrient transformations. Interactions and synergisms within the microbial community are probably essential in soil functions, including decomposition, aggregation, and nutrient release from insoluble reserves, all functions in which fungi and algae play a central role.

## CLASSIFICATION, CHARACTERISTICS, AND ECOLOGICAL ROLES IN SOIL

### FUNGUS-LIKE PROTISTS

A number of unrelated organisms share some of the characteristic features of fungi: they are eukaryotic, nonphotosynthetic chemoheterotrophs that reproduce by spores, many have extracellular digestion and absorptive nutrition, and most have a filamentous growth form. True fungi are further characterized by cell walls containing a mixture of glucan, mannan, and chitin, but not cellulose, and by production

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FIGURE 6.2 (*Continued*)
named sequences. The tree includes only the names of environmental sequences. Major clade names follow Hibbett and Thorn (2001), plus the Ceratobasidiales and Heterobasidiomycetes (Russ, russuloid; G/P, gomphoid/phalloid; Heterobasids, Heterobasidiomycetes). Minor clade names (approximately generic names) follow Moncalvo *et al.* (2002). Occurrence of minor clades in four treatments is shown at right: T1, corn–soybean–wheat rotation with regular tillage and inputs; T2, no-till corn–soybean–wheat rotation with regular inputs; T7, successional site, burned every spring since tillage ceased in 1989; T8, never-tilled native meadow, mown in fall.

of the membrane sterol ergosterol (Alexopoulos *et al.*, 1996). Molecular phylogenetic studies are gradually piecing together the relationships of some of these groups, about which we know very little compared to vascular plants or metazoan animals. Early studies were limited by the small number of representatives in culture, but the growing "Tree of Life" project (e.g., Lutzoni *et al.*, 2004) and broad-scale sequencing of environmental DNA (e.g., Venter *et al.*, 2004) are obtaining sequences for novel taxa and discovering many previous unknown lineages among fungi and basal eukaryotes (e.g., Vandenkoornhuyse *et al.*, 2002; Schadt *et al.*, 2003). Fungus-like members of the "protist" Phylum Cercozoa are represented in soil by soil-borne plant pathogens *Plasmodiophora* and *Spongospora* (Plasmodiophoromycetes), which infect roots via biflagellate zoospores and then form a multinucleate plasmodium within their hosts (Braselton, 2001). Diverse unrecognized lineages of this phylum are apparently widespread (Bass and Cavalier-Smith, 2004). The Oomycota (Heterokonta) include *Pythium* and *Phytophthora*, two genera of important soil-borne plant pathogens with broad host ranges (Erwin *et al.*, 1983; Martin, 1992; Levesque and de Cock, 2004). Infection is via biflagellate zoospores that germinate to form broad, rapidly growing tubular hyphae. Oomycotans differ substantially from true fungi in the chemistry of their cell walls and membranes, particularly the presence of cellulose in cell walls and the absence of ergosterol in membranes and also in ploidy, being diploid rather than haploid or dikaryotic (Fuller and Jaworski, 1987; Dick, 2001). Their flagella include a posterior whiplash flagellum and an anterior tinsel flagellum. The Hyphochytriomycota are a small group of little or no known economic importance; their zoospores have a single anterior tinsel flagellum, but molecular and ultrastructural evidence places them together with the biflagellate heterokont organisms such as Oomycota and chromistan algae (Fuller, 2001). *Hyphochytrium* and *Rhizidiomyces* may be saprotrophs or parasites of fungi or other soil organisms. Molecular evidence places the Labyrinthulomycota together with heterokont protists such as Oomycota (Cavalier-Smith, 2004). *Labyrinthula* have fusoid cells that glide in an extracellular, netlike slime; they are pathogens of marine plants and algae and of vascular plants in saline soils (Bigelow *et al.*, 2005). The Phylum Mycetozoa (=Myxomycota, or slime molds) includes organisms with a mixture of characters resembling fungi and animals: reproduction by spores and ingestion of food by phagocytosis (Baldauf and Doolittle, 1997). Spores of slime molds germinate to form amoebae, which aggregate to form a slime phase that eventually differentiates to form fruiting bodies with spores. In the Class Dictyosteliomycetes (Raper, 1984), the slime phase (pseudoplasmodium) consists of cells that maintain their separate membranes, whereas in the Myxomycetes, the plasmodium is a coenocytic mass of nuclei in a slimy matrix. Both amoeboid and plasmodial phases are phagotrophic on bacteria, spores, and detritus and are probably very important in releasing nutrients that are immobilized in these cells and substrates. Approximately 1000 species in 80 genera are known. Plasmodia and fruiting bodies of some Myxomycetes can be quite conspicuous in lawns and gardens during the summer months (Martin and Alexopoulos, 1969; Stephenson and Stempen, 1994).

## FUNGI (CHYTRIDIOMYCOTA, GLOMEROMYCOTA, ZYGOMYCOTA, ASCOMYCOTA, AND BASIDIOMYCOTA)

The chytrids are a relatively small group of approximately 1000 species in 120 genera (Kirk *et al.*, 2001). Soil chytrids include plant pathogens such as *Synchytrium* and *Olpidium*, nematode parasites such as *Catenaria*, parasites of algae such as *Chytridium*, and saprobes such as *Allomyces* and *Chytriomyces*. The soil chytrids possess motile zoospores powered by a single, posterior flagellum and are thought to be close to the common ancestor of fungi and animals (Mendoza *et al.*, 2002; James *et al.*, 2006). Recognition of the Phylum Glomeromycota (Schuessler *et al.*, 2001) resolves some of the problems seen in earlier phylogenetic analyses in which chytrids formed a monophyletic group that divided the zygomycetes or found that both groups were paraphyletic with respect to the other. Gomeromycota and chytrids share ancient sequences for tubulin genes, remarkably conserved despite the age of their divergence (Corradi *et al.*, 2004). Together, the chytrids, Glomeromycota, and Zygomycota differ from the Ascomycota and Basidiomycota in that they have broad, coenocytic hyphae, with multiple haploid nuclei per cell, whereas the Ascomycota and Basidiomycota typically have uninucleate haploid primary mycelia and binucleate (di-haploid) secondary mycelia. The name Phycomycetes was formerly used to refer to the coenocytic fungi (sometimes together with the unrelated Oomycota). Regrettably, the best general references to these fungi are quite old (e.g., Sparrow, 1960; Karling, 1977), although there has been a resurgence in research on this group (Barr, 2001).

The Glomales (Phylum Glomeromycota) are the fungal symbionts of arbuscular mycorrhizas (also known as endomycorrhizas). They were recently transferred from the Zygomycota to a new phylum on the basis of molecular phylogenetic information, which is supported by their great age (at least 500 My) and unique biology (Schuessler *et al.*, 2001). Broad, multinucleate hyphae extend from plant roots into soil and aid in uptake of water and nutrients, particularly phosphate (Smith and Read, 1997). Large, multinucleate asexual spores (sometimes referred to as chlamydospores or azygospores) are formed in soil or roots, and the morphology of these spores forms almost the sole basis for their taxonomy. Glomalin, a glycoprotein excreted by glomalean fungi, is associated with aggregate stability in soils (Wright and Upadhyaya, 1998; Rillig, 2004). Approximately 150 species in six genera are known (Kirk *et al.*, 2001), and these form symbioses with approximately 70% of all land plants, particularly herbaceous plants and woody plants of the tropics (Brundrett, 2002). Taxonomic and ecological references to the group include Gerdemann and Trappe (1974) and Morton and Benny (1990).

Within the Zygomycota are two very different classes of fungi: the Trichomycetes and Zygomycetes. Trichomycetes (about 200 species) are obligate endosymbionts of arthropods (Benny, 2001; Kirk *et al.*, 2001). Approximately 800 species in eight orders of Zygomycetes are known (Benny *et al.*, 2001; Kirk *et al.*, 2001). Many are saprobes (Mucorales and relatives), and others are parasites of arthropods and other invertebrates or of other fungi (Zoopagales, Entomophthorales,

and Kickxellales). *Rhizopus* and *Mucor* (both Mucorales) are weedy molds readily isolated from soil. In the earlier literature, the saprobic Mucorales were often referred to as "sugar fungi." The accompanying view held that these organisms are rapid colonists making use of the soluble sugars before secondary colonists become established during the succession of different fungi on rich and freshly deposited substrates such as dung and fallen fruits (Garrett, 1963). In reality, the early appearance of Mucorales on such substrates reflects their rapid rates of growth and sporulation; in many cases the "secondary colonists" are already there and digesting the more recalcitrant substrates, but are slower to sporulate and come to our notice (Pugh, 1974; Cooke and Rayner, 1984). Still, the term "sugar fungi" accurately portrays the typical growth substrate of these fungi. They are not noted for their extracellular degradative enzymes and may frequently depend on soluble breakdown products provided by lignocellulose-degrading ascomycetes or basidiomycetes (Cooke and Rayner, 1984). From here, it may have been a short step to direct, biotrophic parasitism of the decomposer fungi, a nutritional mode found among some Mucorales and related groups (O'Donnell, 1979; Benny *et al.*, 2001). The references listed previously provide an entry point for the literature on taxonomy and ecology of soil Zygomycetes.

The Ascomycota are the largest group in number of species (approximately 33,000, plus another 16,000 known only as asexual forms) and span a range of nutritional modes from parasites and pathogens of plants, animals, and other fungi through mutualists (forming both lichens and some ectomycorrhizas) and saprotrophs (Kirk *et al.*, 2001). Meiosis and production of sexual spores (ascospores) occurs within a sac-shaped cell, the ascus. It has often puzzled other biologists that mycologists may refer to the same fungus by two (or more) different names depending on whether it reproduces sexually (referred to as the teleomorph) or asexually (anamorph). A separate, artificial phylum (Deuteromycota, or "Fungi Imperfecti") and a number of artificial class names (Agonomycetes, Coelomycetes, Deuteromycetes, Hyphomycetes) have been used for fungi that lack known sexual structures, but the majority of these are asexual relatives of Ascomycota (Seifert and Gams, 2001). Most fungal systematists have abandoned the Deuteromycota, and many are now trying to unify the classification of these fungi as they are linked by phylogenetic analyses of their DNA sequences. Among the filamentous Ascomycota are many of the most important soil-borne pathogens of crop plants, including wilts caused by *Fusarium* and *Verticillium* and root and stem rots caused by *Cochliobolus, Giberella, Gaeumannomyces, Phymatotrichopsis*, and *Sclerotinia* (Farr *et al.*, 1989; Holliday, 1989; Agrios, 1997). The most familiar and economically important molds, including *Aspergillus* and *Penicillium*, are asexual forms of Ascomycota that are also abundant in soils: *Aspergillus* more commonly in tropical soils and *Penicillium* more commonly in cool temperate and boreal soils (Christensen, 1981). Key literature to the identification of anamorphic and teleomorphic Ascomycota includes Ellis (1971, 1976), Pitt (1979), von Arx (1981), Domsch *et al.* (1993), and Klich (2002), with additional references provided by Kirk *et al.* (2001) and Mueller *et al.* (2004).

Yeasts are fungi adapted to life in aqueous environments often of high osmotic potential, through growth of separate, usually elliptical cells that divide by budding or fission (Barnett *et al.*, 1990; Kurtzman and Fell, 1998). Most yeasts, including the most economically important ones such as *Saccharomyces cerevisiae*, belong in the Ascomycota (Kurtzman and Sugiyama, 2001). Many yeasts are found in association with fruits, flowers, and other rich sources of readily assimilated sugars and other carbohydrates, but others (particularly basidiomycetous yeasts in *Cryptococcus, Rhodosporidium, Rhodotorula*, and *Sporobolomyces*) are found in soil, where they may be closely associated with plant roots or may be important members of the decomposer consortium breaking down plant and animal wastes (Lachance and Starmer, 1998; Fell *et al.*, 2001).

Lichens are symbiotic associations between fungi and green algae or cyanobacteria, in which the fungal partner (mycobiont) forms a characteristic structure called a thallus (what we see as a lichen) that encloses and protects the alga or cyanobacterium (photobiont) (Hale, 1983; Nash, 1996). Just as with mycorrhizal symbioses, lichens evolved independently in the Zygomycota, Ascomycota, and Basidiomycota (Gargas *et al.*, 1995; Lutzoni *et al.*, 2004), but by far the majority of lichen species (over 20,000 described species) are in the Ascomycota (Kirk *et al.*, 2001). Lichens form the dominant ground cover over large areas of arctic and alpine environments. The cryptobiotic soil crusts that protect soils in arid environments may include lichens, together with consortia of fungi (States and Christensen, 2001), algae, and cyanobacteria (West, 1990; Belnap and Lange, 2001). Brodo *et al.* (2001) provide a beautiful and highly useful introduction to lichen identification and biology.

The Basidiomycota differ from Ascomycota primarily by production of sexual spores (basidiospores) outside the basidium, the cell in which meiosis takes place. Approximately 30,000 species are known. The Basidiomycota may be divided into the subphyla Ustilaginomycotina (smuts), Urediniomycotina (rusts), and Hymenomycotina, the latter divided into the Classes Heterobasidiomycetes (jelly fungi) and Homobasidiomycetes (mushrooms and relatives) (modified from Kirk *et al.*, 2001, and McLaughlin and McLaughlin, 2001). Only the last group is important in soil, although various stages of the life cycles of smuts and jelly fungi may occur in soil and be important to their survival. The Homobasidiomycetes, with approximately 13,000 described species, include gilled mushrooms, boletes, polypores, coral fungi, stinkhorns, and crust fungi, many with their mycelial phase occurring in soil (Hibbett and Thorn, 2001). This great diversity has mostly been overlooked in surveys of soil fungi. Within the Homobasidiomycetes are some very important soil-borne pathogens of crop plants, including *Rhizoctonia* (sexual states in *Thanatephorus* and several other genera; Roberts, 1999), and of forest trees, including *Armillaria, Phellinus*, and *Ganoderma* (Tainter, 1996). The majority of Homobasidiomycetes (perhaps 8500 described species) are saprotrophic leaf- and wood-decomposing fungi; their activities may extend well into the mineral horizons of soil wherever organic materials are present (Rayner and Boddy, 1988; Hibbett and Thorn, 2001).

Approximately 4500 described species of Homobasidiomycetes form ectomycorrhizal relations with woody vascular plants in 30 families. Forests of ectomycorrhizal trees are dominant over much of the temperate, boreal, and alpine regions of the world, whereas tropical forests are mostly dominated by species with arbuscular mycorrhizas, with ectomycorrhizal trees widely dispersed or locally dominant (Smith and Read, 1997; Brundrett, 2002). Boreal forests with very few species of trees (perhaps 2 or 3 dominants) form ectomycorrhizas with hundreds or even thousands of species of fungi. By contrast, highly diverse tropical forests (with 80–100 species per hectare) form arbuscular mycorrhizas with a handful of morphospecies of Glomales. Each of these represents an evolutionary conundrum.

Homobasidiomycetes also include nematode-destroying fungi, fungi cultivated by leaf-cutting ants and their relatives, and "corpse-finder fungi" that fruit near animal corpses or accumulations of dung or urine (Thorn, 2002). Their tremendous range of nutritional modes and their persistent, extensive mycelia make Homobasidiomycetes of great importance in spanning space, time, and trophic levels within terrestrial ecosystems. There is a tremendous literature for identification of mushrooms and other Homobasidiomycetes, among which Moser (1983), Gilbertson and Ryvarden (1986–1987), Jülich and Stalpers (1980), and Stalpers (1993, 1996) are good starting points for temperate taxa, with additional references provided in Kirk *et al.* (2001) and Mueller *et al.* (2004). Unfortunately, there are few morphological characters and no useful references for identifying cultures of these fungi isolated from soil; the best hope lies in the growing database of ribosomal DNA sequences.

## EUKARYOTIC ALGAE

The temporarily or permanently wet margins of rivers, lakes, and ponds and the peaty substrates of bogs and fens are home to many freshwater algae that do not thrive far from water. These may be referred to as the hydroterrestrial algae. However, the diverse soils of temperate and tropical agriculture, grasslands, forests, arctic and alpine tundra, and even deserts also harbor a surprisingly diverse and active flora of both eukaryotic algae and cyanobacteria. These are referred to as terrestrial algae, or sometimes as "edaphic algae." Cryptobiotic crusts (formed by eukaryotic algae together with filamentous fungi, yeasts, and cyanobacteria) are termed "epedaphic." General discussion or conclusions regarding the algal flora of soil and its functioning are hampered by historic taxonomic confusion and by literature up to the present day in which the numbers and activities of cyanobacteria are included together with those of eukaryotic algae. The roles of these two groups in the soil nitrogen cycle are vastly different, since common soil cyanobacteria such as *Nostoc, Anabaena, Tolypothrix, Scytonema*, and *Cylindrospermum* are highly active in nitrogen fixation, with rates often 20–30 kg $ha^{-1}$ $year^{-1}$ (Zackrisson *et al.*, 2004); rates in desert soil crusts are lower (1.4–9 kg $ha^{-1}$ $year^{-1}$) and dependent upon taxonomic composition, moisture, and suitable temperatures (Belnap, 2002). There is room for a great deal of research to tease out the particular roles and quantitative significance of the various eukaryotic algae in soil.

However, in general, it can be said that they play an important role in net primary production and the incorporation of organic carbon and nitrogen into soil, crucial during primary succession on land created by volcanic activity or bared by the retreat of glaciers. Upon their death, much of this organic matter is generally readily accessible to microbial decomposition and enters the actively cycling nutrient pool. A small part, made up of cell wall materials and exudates, may become complexed with soil phenolics of plant origin and form more stable humic substances that help to provide soil structure. Together with soil fungi and some other microbes, terrestrial algae may also be responsible for liberation of nutrients from insoluble sources in the mineral fraction or horizon of soil (Metting, 1981; Jongmans *et al.*, 1997). Finally, in many dune or desert environments where vascular plant growth is restricted by permanent or seasonal drought, eukaryotic algae alone or in mixed cryptobiotic crusts play a vital role in stabilizing the "soil" surface, gradually adding soil organic matter that holds soil moisture and provides nutrients for plant establishment.

Soil is a common habitat for nonmotile green algae. Both filamentous and coccoid forms occur, the latter more common in desert soils. Green algae tend to dominate the algal flora of acid soils, and some are observed only following enrichment culturing. Frequent genera include *Actinochloris, Ankistrodesmus, Bracteacoccus, Characium, Chlorella, Chlorococcum, Chlorosarcinopsis, Fernandinella, Hormotilla, Keratococcus, Muriella, Protosiphon, Stichococcus, Tetracystis, Apatococcus, Desmococcus, Klebsormidium*, and *Ulothrix*. Most are limited to the surface of wet soils, and some are epiphytic on algae or mosses. *Trentepohlia* forms long, brilliant orange filaments on soil-free rocks and bark, often not recognized as a green alga even by those who notice it. In this and many other Chlorophyta, photoprotective carotenoids mask the "typical" grass green color.

Red algae are infrequent in typical soils. Species of *Cyanidium* are found in and around acidic hot springs and species of *Porphyridium* grow as reddish gelatinous masses encrusting polluted, ammonium-rich soils in shaded areas and on wet, well-decayed logs. Both are spherical unicells with a mucilaginous matrix.

Species of *Euglena* (fusoid, uniflagellate cells with red eyespot and with or without chloroplasts) are abundant where moisture, organic matter, and often ammonium are high, such as wet footprints in paddocks, puddles, ditches, and farm ponds. Facultative photoautotrophs, they can subsist as achlorophyllous heterotrophs. The nonphotosynthetic, phagotrophic *Peranema* also frequents similar habitats.

Members of the golden-green algae (Xanthophyta) are usually found on the surface of moist soils. Vegetative cells are nonmotile and exhibit a variety of growth forms, from unicellular and globose or cylindrical to colonial and coenocytic, or filamentous. Genera reported from soil include *Botrydiopsis, Botrydium, Bumilleria, Bumilleriopsis, Heterococcus, Vaucheria,* and *Xanthonema* (=*Heterothrix*).

Diatoms form beautiful exoskeletons (frustules) of silica; deposits of millennia form diatomaceous earth, which can be used as a natural insecticide. Marine diatoms are probably the single largest primary producers in the world, and diatoms are also abundant in freshwater ecosystems. In terrestrial ecosystems, diatoms

occur primarily in neutral to slightly alkaline soils, where their populations may reach $10^5$ cells per gram dry weight of soil (Bérard *et al.*, 2004). Members of the genera *Achnanthes, Cymbella, Fragilaria, Hantzschia, Navicula, Pinnularia, Stauroneis*, and *Surirella* (many of which are admittedly artificial) are frequently reported. These are organisms of moist surfaces, including plants, other algae, litter, and bare mineral or organic soil.

For reviews of terrestrial algae, see Metting (1981) and Hoffmann (1989); useful taxonomic references include Foged (1978), Dodd (1987), Entwisle *et al.* (1997), Ling and Tyler (2000), John *et al.* (2002), and Wehr and Sheath (2003). As with much of biology, much more is known about these organisms in temperate (especially north temperate) regions of the world, but the biological roles of terrestrial algae everywhere deserve considerably more study.

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# 7

# FAUNA: THE ENGINE FOR MICROBIAL ACTIVITY AND TRANSPORT

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## INTRODUCTION

Animals, another group of major heterotrophs in soil systems, can be viewed as facilitators of bacterial and fungal activity and diversity in soils. They exist in food webs containing several trophic levels. Some are herbivores, since they feed directly on roots of living plants, but most subsist upon dead plant matter (saprophytes), or living microbes associated with it, or a combination of the two. Still others are carnivores, parasites, or top predators. Analyses of food webs in the soil have emphasized numbers of the various organisms and their trophic resources. The structure of these food webs is complex, with many "missing links" poorly described or as yet unknown (Walter *et al.*, 1991; Scheu and Setälä, 2002).

Animal members of the soil biota are numerous and diverse and include representatives of all terrestrial phyla. Many groups of species are not described taxonomically, and details of their natural history and biology are unknown. For the microarthropods only about 10% of populations have been explored and perhaps 10% of species described (André *et al.*, 2002). We feel protection of biodiversity in ecosystems clearly must include the rich pool of soil species. This is because data for some of these species individually and collectively indicate tight connections to biodiversity aboveground, major roles in ecosystem processes, and provision of ecosystem benefits for human well-being (Wardle *et al.*, 2004; Wall *et al.*, 2005; Wall, 2004).

When research focuses at the level of the soil ecosystem two things are required: the cooperation of multiple disciplines (soil scientists, zoologists, and microbiologists) and the lumping of animals into functional groups. These groups are often taxonomic, but species with similar biologies and morphologies are grouped together for purposes of integration (Coleman *et al.*, 1993; Hendrix *et al.*, 1986; Hunt *et al.*, 1987).

The soil fauna also may be characterized by the degree of presence in the soil or microhabitat utilization by different life forms. There are transient species, exemplified by the ladybird beetle, which hibernates in the soil but otherwise lives in the plant stratum. Gnats (Diptera) are temporary residents of the soil, since the adult stages live aboveground. Their eggs are laid in the soil and their larvae feed on decomposing organic debris. In some soil situations dipteran larvae are important scavengers. Cutworms are temporary soil residents, whose larvae feed on seedlings by night. Nematodes that parasitize insects and beetles spend part of their life cycle in soil. Periodic residents spend their life histories belowground, with adults, such as the velvet mites, emerging to reproduce. The soil food webs are linked to aboveground systems, making trophic analyses much more complicated than in either subsystem alone (Wardle *et al.*, 2004). Even permanent residents of the soil may be adapted to life at various depths in the soil.

Among the microarthropods, collembolans are examples of permanent soil residents. The morphology of collembolans reveals their adaptations for life in different soil strata. Species that dwell on the soil surface or in the litter layer may be large, pigmented, and equipped with long antennae and a well-developed jumping apparatus (furcula). Collembolans living within mineral soil tend to be smaller, with unpigmented, elongate bodies, and possess a much reduced furcula.

A generalized classification by length illustrates a commonly used device for separating the soil fauna into size classes: microfauna, mesofauna, macrofauna, and megafauna. This classification encompasses the range from smallest to largest, i.e., from ca. 1–2 μm for the microflagellates to >2 m for giant Australian earthworms. Body width of the fauna is related to their microhabitats (Fig. 7.1). The microfauna (protozoa, rotifers, tardigrades, small nematodes) inhabit water films. The mesofauna inhabit existing air-filled pore spaces and are largely restricted to existing spaces. The macrofauna have the ability to create their own spaces, through their burrowing activities, and like the megafauna, can have large influences

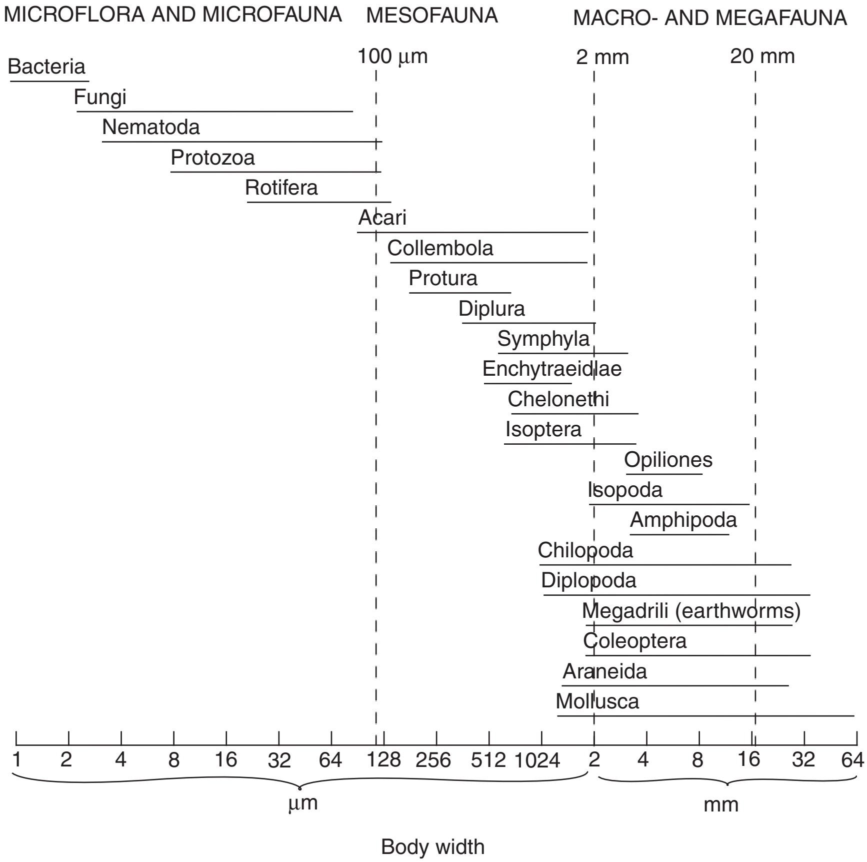



FIGURE 7.1 Size classification of organisms in decomposer food webs by body width (Swift *et al.*, 1979).

on gross soil structure (Lavelle and Spain, 2001; van Vliet and Hendrix, 2003). Methods for studying these faunal groups are mostly size-dependent. The macrofauna may be sampled as field collections, often by hand sorting, and populations of individuals are usually measured.

There is considerable gradation in the classification based on body width. The smaller mesofauna exhibit characteristics of the microfauna, and so forth. The vast range of body sizes among the soil fauna emphasizes their effects on soil processes at a range of spatial scales. Three levels of participation have been suggested (Lavelle *et al.*, 1995; Wardle, 2002). "Ecosystem engineers," such as earthworms, termites, or ants, alter the physical structure of the soil itself, influencing rates of nutrient and energy flow. "Litter transformers," the microarthropods, fragment decomposing litter and improve its availability to microbes. "Micro-food webs" include the microbial groups and their direct microfaunal predators (nematodes

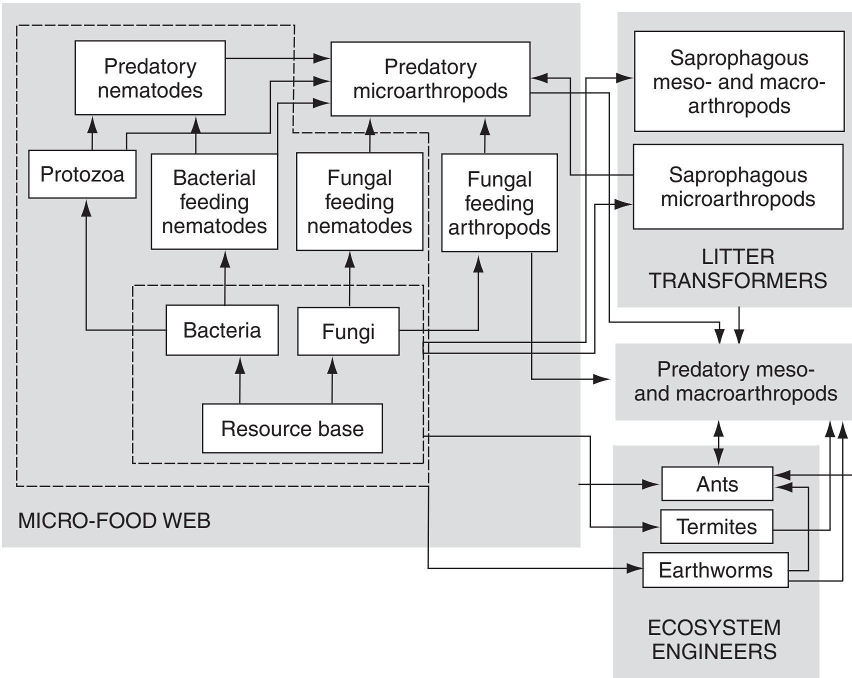



FIGURE 7.2 Organization of the soil food web into three categories—ecosystem engineers, litter transformers, and micro-food webs (after Wardle, 2002, and Lavelle *et al.*, 1995).

and protozoans). These three levels operate on different size, spatial, and time scales (Fig. 7.2, Wardle, 2002).

## THE MICROFAUNA

The free-living protozoa of litter and soils belong to two phyla, the Sarcomastigophora and the Ciliophora. They are considered in four ecological groups: the flagellates, naked amoebae, testacea, and ciliates (Lousier and Bamforth, 1990). A general comparison of body plans is given in Fig. 7.3, showing representatives of the four major types.

1. *Flagellates* (named for their one or more flagella, or whip-like propulsive organs) are among the more numerous and active of the protozoa. They play a significant role in nutrient turnover, with bacteria as their principal prey items (Zwart and Darbyshire, 1991; Kuikman and Van Veen, 1989). Numbers vary from $10^2\,g^{-1}$ in desert soils to more than $10^5\,g^{-1}$ in forest soils (Bamforth, 1980).

2. *Naked amoebae* are among the more voracious of the soil protozoa and are very numerous and active in a wide range of agricultural, grassland, and forested soils (Clarholm, 1981, 1985; Gupta and Germida, 1989). The dominant mode of

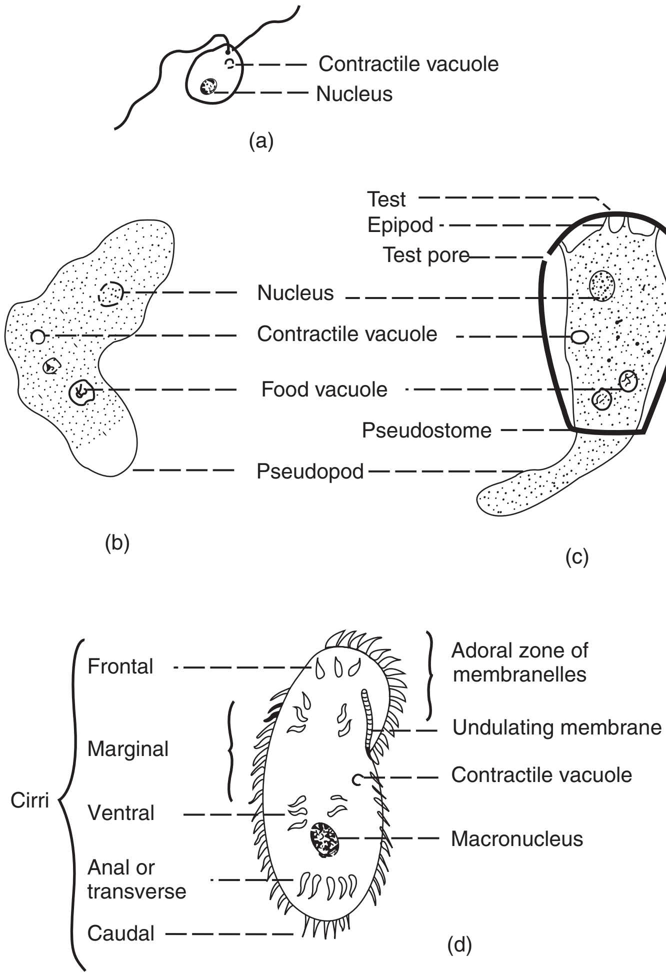



FIGURE 7.3 Morphology of four types of soil Protozoa: (a) flagellate (*Bodo*); (b) naked amoeba (*Naegleria*); (c) testacean (*Hyalosphenia*); (d) ciliate (*Oxytricha*) (from Lousier and Bamforth, 1990).

feeding for the amoebae, as for the larger forms such as Ciliates, is phagotrophic (engulfing), with bacteria, fungi, algae, and other fine particulate organic matter being the majority of the ingested material. They are highly plastic, in terms of their ability to explore very small cavities or pores in soil aggregates and to feed upon bacteria that would otherwise be considered inaccessible to predators (Foster and Dormaar, 1991).

3. *Testate amoebae:* Compared with the naked amoebae, testate amoebae are often less numerous, except in moist, forested systems. They are more easily enumerated by a range of direct filtration and staining procedures. Lousier and Parkinson (1984) noted a mean annual biomass of 0.07 g dry wt $m^{-2}$ of aspen

woodland soil, much smaller than the average annual mass for bacteria or fungi, 23 and 40 g dry wt $m^{-2}$, respectively. However, the testacean annual secondary production (new tissue per year) was 21 g dry wt $m^{-2}$, which they calculated to be essentially the entire average standing crop of the bacteria in that site.

4. *Ciliates* have unusual life cycles and complex reproductive patterns and tend to be restricted to very moist or seasonally moist habitats. Their numbers are lower than those of other groups, with a general range of 10 to 500 $g^{-1}$ of litter/soil. Ciliates can be very active in entering soil cavities and pores and exploiting bacterial food sources (Foissner, 1987). Ciliates, like other protozoa, have resistant or encysted forms and emerge when conditions, such as food availability, are favorable for growth and reproduction. Ciliates, flagellates, and naked and testate amoebae reproduce asexually by fission. The flagellates, naked amoebae, and testacea reproduce by syngamy, or fusion of two cells. For the ciliates, sexual reproduction occurs by conjugation, with the micronucleus undergoing meiosis in two individuals and the two cells joining at the region of the cytostome and exchanging haploid "gametic" nuclei. Each ciliate cell then undergoes fission to produce individuals, which are genetically different from the preconjugant parents (Lousier and Bamforth, 1990).

## METHODS FOR EXTRACTING AND COUNTING PROTOZOA

Researchers have favored the culture technique (Singh, 1946), in which small quantities of soil or soil suspensions from dilution series are incubated in small wells, inoculated with a single species of bacteria as a food source. Based on the presence or absence in each well, one can calculate the overall population density ("most probable number"). Adl (2003), Coûteaux (1972), and Foissner (1987) favor the direct count approach, in which one examines soil samples, in water, to see the organisms present in the subsample. The advantage of direct counting is that it is possible to observe the organisms immediately present and not rely on the palatability of the bacterium as substrate in the series of wells in the culture technique. The disadvantage of the direct count method is that one usually employs only 5–30 mg of soil, so as not to be overwhelmed with total numbers (Foissner, 1987). This discriminates against some of the more rare forms of testaceans or ciliates that may have a significant impact on an ecosystem process, if they happen to be very large.

The culture technique attempts to differentiate between active (trophozoite forms) and inactive (cystic) forms by treatment of replicate samples with 2% hydrochloric acid overnight. The acid kills off the trophozoite forms. After a wash in dilute NaCl, the counting continues. This assumes that all cysts will excyst after this drastic process, an assumption not always met.

## IMPACTS OF PROTOZOA ON ECOSYSTEM FUNCTION

Several investigators have noted the obvious parallel between the protozoan–microbe interaction in water films in soil and on root surfaces and in open-water aquatic systems (Stout, 1963; Clarholm, 1994; Coleman, 1994). The "microbial

loop," defined by Pomeroy (1974) as the rapid recycling of nutrients by protozoan grazers, is a powerful conceptual tool. In a fashion similar to that occurring in aquatic systems, rapidly feeding protozoa may consume one or more standing crops of bacteria in soil every year (Clarholm, 1985; Coleman, 1994). This tendency is particularly marked in the rhizosphere, which provides a ready food source for microbial prey.

The population dynamics of bacteria, naked amoebae, and flagellates were followed in the humus layer of a pine forest in Sweden (Clarholm, 1994). Bacteria and flagellates began increasing in number immediately after a rainfall event and rose to a peak after 2–3 days. Naked amoebae rose more slowly, peaked after 4–5 days, and then tracked the bacterial decrease downward. The flagellates followed a pattern similar to that of the naked amoebae. Further information on protozoan feeding activities and their impacts on other organisms and ecosystem function is given in Darbyshire (1994). Bonkowski *et al.* (2000) suggest that protozoa, and the bacteria they feed upon in the rhizosphere, produce plant growth-promoting compounds that stimulate plant growth above and beyond the amounts of N mineralized in the rhizosphere. Protozoa and other microfauna are quite sensitive to environmental insults, and changes in the distribution and activities are diagnostic of changes in soil health (Gupta and Yeates, 1997).

### DISTRIBUTION OF PROTOZOA IN SOIL PROFILES

Although protozoa are distributed principally in the upper few centimeters of a soil profile, they are also found at depth, over 200 m deep in groundwater environments (Sinclair and Ghiorse, 1989). Small (2–3 μm cell size) microflagellates decreased 10-fold in numbers during movement through 1 m in a sandy matrix under a trickling-filter facility (in dilute sewage), compared to a 10-fold reduction in bacterial transport over a 10-m distance (Harvey *et al.*, 1995).

## ROTIFERA

These small fauna are often found only when a significant proportion of water films exist in soils. While they may not be listed in major compendia of soil biota (Dindal, 1990), they are a genuine, albeit secondary, component of the soil fauna (Wallwork, 1976). Rotifers exist in bagged leaf litter and in even more extreme environments, such as the soils of the Antarctic Dry Valleys (Treonis *et al.*, 1999).

More than 90% of soil rotifers are in the Order Bdelloidea, or worm-like rotifers. In these creeping forms, the suctorial rostral cilia and the adhesive disc are employed for locomotion (Donner, 1966). Rotifers, like tardigrades and nematodes, can enter a desiccated resistant state (anhyrobiosis) at any stage in their life cycle, in response to environmental stress. When the stress is removed, they rehydrate and become active. Additional life history features of interest include the construction of shells from a body secretion, which may have particles of debris

and/or fecal material adhering to it. Some rotifers will use the empty shells of Testacea, the thecate amoebae, to survive. The Bdelloidea are vortex feeders, creating currents of water that conduct food particles, such as unicellular algae or bacteria, to the mouth for ingestion. The importance of these organisms is largely unknown, although they may reach numbers exceeding $10^5\ m^{-2}$ in moist, organic soils (Wallwork, 1970). Rotifers are extracted from soil samples and enumerated using methods similar to those used for nematodes (see the following).

## NEMATODA

The Phylum Nematoda contains nematodes or roundworms, which are among the most numerous and diverse of the multicellular organisms found in any ecosystem. It has been estimated that four of every five animals on earth are nematodes (Bongers and Ferris, 1999). As with the protozoa, rotifers, and tardigrades, nematodes live in water films or water-filled pore spaces in soils. Nematodes have a very early phylogenetic origin among the Eukarya (Blaxter *et al.*, 1998), but as with other invertebrate groups, the fossil record is fragmentary. Nematodes are most closely related to the Rotifers, Gastrotrichs, and Nematophora. They are considered to be triploblastic pseudocoelomates (three body layers: ectoderm, mesoderm, and endoderm).

The overall body shape is cylindrical, tapering at the ends (Fig. 7.4). Nematode body plans are characterized by a "tube within a tube" (alimentary tract/the body wall). They have a complete digestive system or an alimentary tract, consisting of a stoma or stylet, pharynx (or esophagus), and intestine and rectum, which opens externally at the anus. The reproductive structures are complex, and sexes are generally dimorphic. Some species are parthenogenetic, producing only females. Nematodes are highly diverse but can be identified to order or family by examining specific morphological characteristics under high magnification ($>100\times$) using compound microscopes.

### NEMATODE FEEDING HABITS

Nematodes feed on a wide range of foods. A general trophic grouping is: bacterial feeders, fungal feeders, plant feeders, and predators and omnivores. For the purposes of our overview, one can use anterior (stomal or mouth) structures to differentiate feeding, or trophic, groups (Fig. 7.5) (Yeates and Coleman, 1982; Yeates *et al.*, 1993). Plant-feeding nematodes have a hollow stylet that pierces cell walls of higher plants. Some species are facultative, feeding occasionally on plant roots or root hairs. Others, more recognized for their damage to agricultural crops and forest plantations, are obligate parasites of plants and feed internally or externally on plant roots. The effect nematodes have on plants is generally species-specific and can include alterations in root architecture, water transport, and plant metabolism, or all of these. Recently, the sedentary obligate parasites were found to

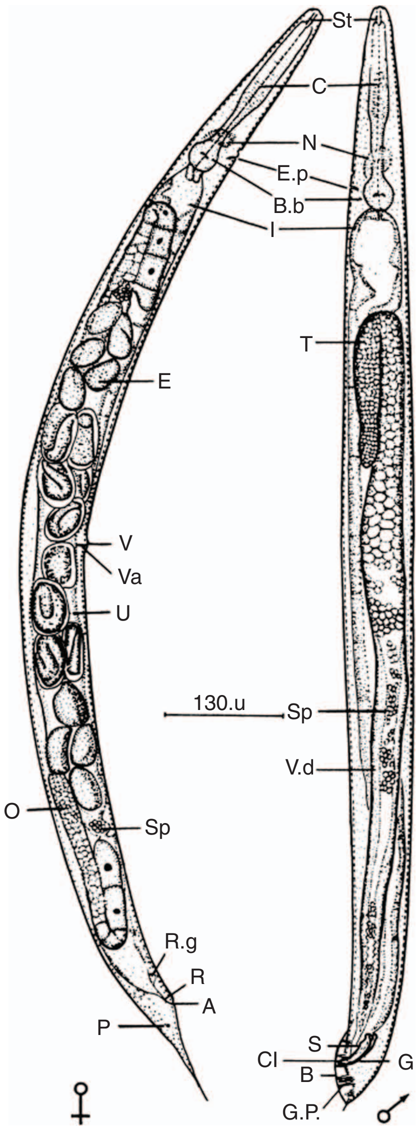



FIGURE 7.4 Structures of a *Rhabditis* sp., a secernentean microbotrophic nematode of the order Rhabditida. (Left) Female. (Right) Male. St, stoma; C, corpus area of the pharynx; N, nerve ring; E.p, excretory pore; B.b, basal bulb of the pharynx; I, intestine; T, testis; E, eggs; V, vulva; Va, vagina; U, uterus; O, ovary; Sp, sperm; V.d, vas deferens; R.g, rectal glands; R, rectum; A, anus; S, spicules; G, gubernaculum; B, bursa; P, phasmids; G.P., genital papillae; Cl, cloaca (courtesy of *Proceedings of the Helminthological Society of Washington*) (from Poinar, 1983).

have multiple parasitic genes. Some of the genes for secretion of endogluconases (cellulases) appear to play direct roles in the nematode parasitic process. Their enzyme products modify plant cell walls and cell metabolism (Davis *et al.*, 2000, 2004). These genes have greatest similarity to microbial genes for cellulases, but

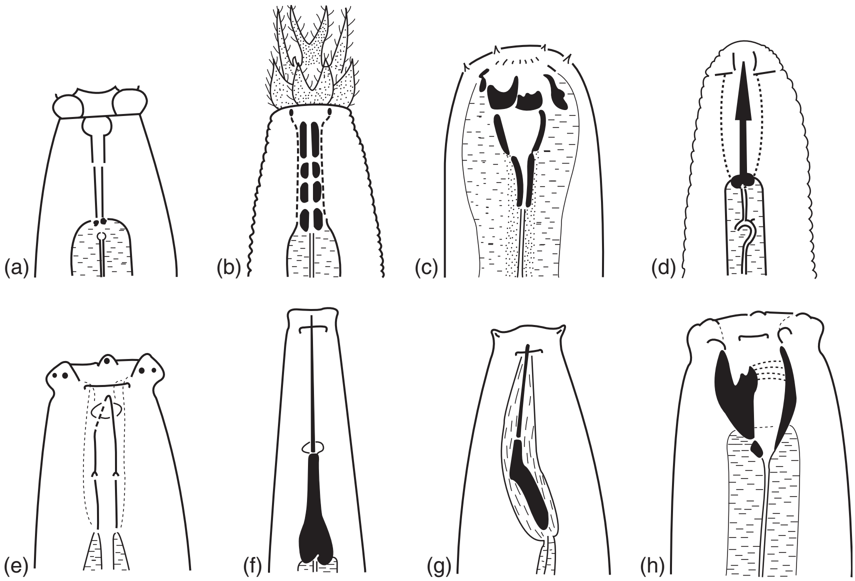



FIGURE 7.5 Head structures of a range of soil nematodes. (a) *Rhabditis* (bacterial feeding); (b) *Acrobeles* (bacterial feeding); (c) *Diplogaster* (bacterial feeding, predator); (d) tylenchid (plant feeding, fungal feeding, predator); (e) *Dorylaimus* (feeding poorly known, omnivore); (f) *Xiphinema* (plant feeding); (g) *Trichodorus* (plant feeding); (h) *Mononchus* (predator) (from Yeates and Coleman, 1982).

coevolution of plant and parasite seems more likely than horizontal gene transfer from microbes to parasite.

Some of the stylet-bearing nematodes (e.g., the Family Neotylenchidae) may feed on roots, root hairs, and fungal hyphae (Yeates and Coleman, 1982). Some bacterial feeders (e.g., Alaimus) may ingest 10-μm-wide cyanobacterial cells (Oscillatoria) despite the mouth of the nematode being only 1 μm wide. This indicates that the cyanobacterial cells can be compressed markedly by the nematode (Yeates, 1998). The population growth of bacterial-feeding nematodes is strongly dependent on the species of bacteria ingested (Venette and Ferris, 1998). Immature forms of certain nematodes may be bacterial feeders and then become predators or parasites on other fauna once they have matured.

The feeding habits and impacts of entomopathogenic nematodes, nematodes carrying symbiotic bacteria that are lethal to their insect host, are distributed worldwide. They have been cultured and sold commercially to control garden pests and mosquitoes (Gaugler, 2002; Hominick, 2002). The nonfeeding, infective juveniles, or third instar (dauer) larvae, of nematodes in the Family Heterorhabditidae and Steinernematidae, live in the soil and search for insect hosts (Gaugler, 2002). The infective juvenile enters the insect host (which it senses along a $CO_2$ gradient

(Strong *et al.*, 1996)) through a body opening, punctures a membrane, and releases its symbiotic bacteria, which kill the host within 24–48 h. A rapidly growing bacterial population then digests the insect cadaver and provides food for the exponentially growing adult nematode population. The symbiotic bacteria produce antibiotics and other antimicrobial substances that protect the host cadaver and adult nematodes inside from invasion by alien bacteria and fungi from the soil (Strong *et al.*, 1999). When the cadaver is exhausted of resources, reproduction shunts to infective juveniles, which break through the host integument and disperse into the soil. As many as 410,000 *Heterorhabditis hepialus* infective juveniles are produced in a large ghost moth caterpillar.

Ruess *et al.* (2002) have traced the fatty acids specific to fungi to the body tissues of fungal-feeding nematodes. This technique shows considerable promise for more detailed biochemical delineation of food sources of specific feeding groups of nematodes. Fungal-feeding nematodes are known to feed preferentially on different fungal species (Mankau and Mankau, 1963), including mycorrhizas and yeasts. Because of the wide range of feeding types and the fact that they seem to reflect ages of the systems in which they occur, i.e., annual vs perennial crops, old fields and pastures, and more mature forests, nematodes have been used as indicators of overall ecological condition (Bongers, 1990; Freckman and Ettema, 1993; Ferris *et al.,* 2001). This is a growing area of research in soil ecology, one in which the intersection between community analysis and ecosystem function could prove to be quite fruitful.

## ZONES OF NEMATODE ACTIVITY IN SOIL

The soil fauna may be concentrated in the rhizosphere. Ingham *et al.* (1985) found up to 70% of the bacterial- and fungal-feeding nematodes in the 4–5% of the total soil that was rhizosphere, namely the amount of soil 1–2 mm from the root surface (the rhizoplane). Griffiths and Caul (1993) found that nematodes migrated to packets of decomposing grass residues, where there were considerable amounts of labile substrates and microbial food sources. They concluded that nematodes seek out these “hot spots” of concentrated organic matter and that protozoa do not. Nematodes also move and occur vertically in soils. In deserts, nematodes are associated with plant roots to depths of 15 m as are mites and other biota (Freckman and Virginia, 1989).

Wallace (1959) noted that movement of nematodes was optimum when soil pores were half drained of free water. Using pressure plates, Demeure *et al.* (1979) showed that nematode movement was found to cease and anhydrobiosis to begin when the water film thickness surrounding a soil particle is between 6 and 9 monomolecular layers of water. This is equivalent to soil pores being completely drained of free water. However, a nematode species from desert habitats tolerated drier soils with less pore water than a species from a tropical habitat. Elliott *et al.* (1980) noted that the limiting factor for nematode survival often hinges on the availability and size of soil pore necks, which enable passage between soil pores. Yeates *et al.* (2002) measured the movements, growth, and survival of three genera of

bacterial-feeding soil nematodes in undisturbed soil cores maintained on soil pressure plates. Interestingly, the nematodes showed significant reproduction even when diameters of water-filled pores were approximately 1 μm.

### NEMATODE EXTRACTION TECHNIQUES

Nematodes may be extracted by a variety of techniques, either active or passive in nature. The principal advantage of the oldest, active method, namely the Baermann funnel method, is that it is simple, requiring no sophisticated equipment or electricity. It is based on the animal's movement and gravity. Samples are placed on coarse tissue paper, on a coarse mesh screen, and then placed in the cone of a funnel and immersed in water. After crawling through the moist soil and filter paper, the nematodes fall down into the neck of the funnel and fall to the bottom of the funnel stem, which is closed off with a screw clamp on a rubber hose. At the conclusion of the extraction (typically 48 h), the nematodes in solution are drawn off into a vial and kept preserved for examination later. Drawbacks to the technique are that only active nematodes are extracted. It also allows dormant nematodes to become active and eggs to hatch into juveniles and be extracted, yielding a slightly inflated estimate of the true, "active" population at a given time. For more accuracy in determination of populations, the passive, or flotation, techniques are generally preferred. Passive methods include filtration, or decanting and sieving, and flotation/centrifugation (Coleman *et al.*, 1999) to remove the nematodes from the soil suspension. Elutriation methods can be employed for handling larger quantities of soil, usually greater than 500 g, or to recover large amounts and a greater diversity of nematodes. Elutriation methods rely on fast mixing of soil and water in funnels. Semiautomatic elutriators, which enhance the number of soil samples to be extracted, are available (Byrd *et al.*, 1976). There are many references comparing methods, including McSorley and Frederick (2004), Schouten and Arp (1991), and Whitehead and Hemming (1965). Anhydrobiotic nematodes can be extracted in a high-molarity solution such as sucrose, which prevents the nematodes from rehydrating (Freckman *et al.*, 1977).

## MICROARTHROPODS

Large numbers of the microarthropod group (mainly mites and collembolans) are found in most types of soils. A square meter of forest floor may contain hundreds of thousands of individuals representing thousands of species. Microarthropods have a significant impact on the decomposition processes in the forest floor and are important reservoirs of biodiversity in forest ecosystems. Many microarthropods feed on fungi and nematodes, thereby linking the microfauna and microbes with the mesofauna. Microarthropods in turn are prey for macroarthropods, such as spiders, beetles, ants, and centipedes, thus bridging a connection to the macrofauna.

In the size spectrum of soil fauna, the mites and collembolans are mesofauna. Members of the microarthropod group are unique, not so much because of their

body size as because of the methods used for sampling them. Small pieces of habitat (soil, leaf litter, or similar materials) are collected and the microarthropods extracted from them in the laboratory. Most of the methods used for microarthropod extraction are either variations of the Tullgren funnel, which uses heat to desiccate the sample and force the arthropods into a collection fluid, or flotation in solvents or saturated sugar solutions followed by filtration (Edwards, 1991). Generally, flotation methods work well in low organic, sandy soils, while Tullgren funnels perform best in soils with high organic matter content. Flotation procedures are more laborious than the Tullgren extraction. Better estimates of species number may be achieved using fewer, larger samples. However, valid comparisons of microarthropod abundance in different habitats may be obtained even if extraction efficiencies, though unknown, are similar.

Microarthropod densities vary during seasons within and between different ecosystems. Generally, temperate forest floors with large organic matter content support high numbers ($33–88 \times 10^3\,m^{-2}$) and coniferous forests may have in excess of $130 \times 10^3\,m^{-2}$. Tropical forests, where the organic layer is thin, contain fewer microarthropods (Seastedt, 1984; Coleman *et al.*, 2004). Tillage, fire, and pesticide applications typically reduce populations, but recovery may be rapid and microarthropod groups respond differently. Soil mites usually outnumber collembolans but the latter become more abundant in some situations. In the springtime, forest leaf litter may develop large populations of "snow fleas" (*Hypogastrura nivicola* and related species). Among the mites themselves, the oribatids usually dominate but the delicate Prostigmata may develop large populations in cultivated soils with a surface crust of algae. Estimation of species richness is a difficult problem for most soil fauna, including ants.

## ENCHYTRAEIDS

In addition to earthworms (discussed under Macrofauna), another important family of terrestrial Oligochaeta is the Enchytraeidae. This group of small, unpigmented worms, also known as "potworms," is classified within the "microdrile" oligochaetes and consists of some 600 species in 28 genera. Species from 19 of these genera are found in soil, the remainder occur primarily in marine and freshwater habitats (Dash, 1990; van Vliet, 2000). The Enchytraeidae are thought to have arisen in cool temperate climates where they are commonly found in moist forest soils rich in organic matter. Various species of enchytraeids are now distributed globally from subarctic to tropical regions.

Keys to the common genera were presented by Dash (1990). Identification of enchytraeid species is difficult, but genera may be identified by observing internal structures through the transparent body wall of specimens mounted on slides.

The Enchytraeidae are typically 10–20 mm in length and are anatomically similar to the earthworms, except for the miniaturization and rearrangement of features overall. They possess setae (with the exception of one genus), and a clitellum in

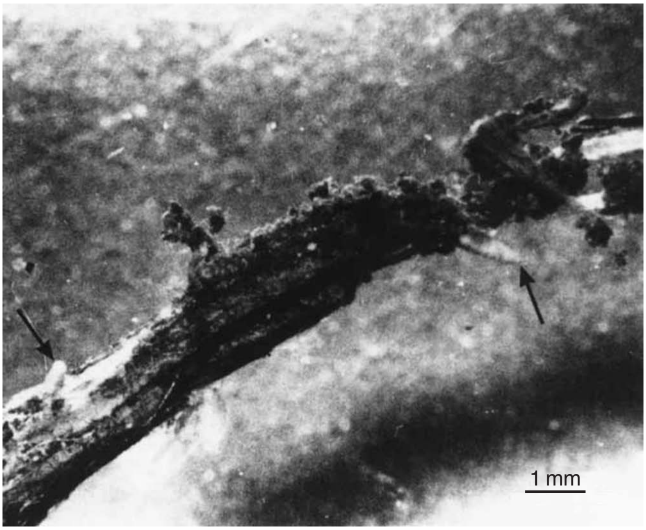



FIGURE 7.6 Two enchytraeid worms tunneling through a pine needle, indicated by arrows (fecal pellets have been deposited on the outside). F1 layer (modified from Ponge, 1991).

segments XII and XIII, which contains both male and female pores. Sexual reproduction in enchytraeids is hermaphroditic and functions similarly to that in earthworms. Cocoons may contain one or more eggs, and maturation of newly hatched individuals ranges from 65 to 120 days depending on species and environmental temperature (van Vliet, 2000). Enchytraeids also display asexual strategies of parthenogenesis and fragmentation, which enhance their probability of colonization of new habitats (Dósza-Farkas, 1996).

Enchytraeids ingest both mineral and organic particles in the soil, although typically of smaller size ranges than those of earthworms. Numerous investigators have noted that finely divided plant materials, often enriched with fungal hyphae and bacteria, are a principal portion of the diet of enchytraeids. Microbial tissues are probably the fraction most readily assimilated because enchytraeids lack the gut enzymes to digest more recalcitrant soil organic matter (van Vliet, 2000). Didden (1990, 1993) suggested that enchytraeids feed predominantly upon fungi, at least in arable soils, and classified a community as 80% microbivorous and 20% saprovorous. As with several other members of the soil mesofauna, the mixed microbiota that occur on decaying organic matter, either litter or roots, are probably an important part of the diet of these creatures. The remaining portions of organic matter, after the processes of ingestion, digestion, and assimilation, become part of the slow-turnover pool of soil organic matter. Zachariae (1964) suggested that so-called "collembolan soil," said to be dominated by collembolan feces (particularly low-pH mor soils), were actually formed by Enchytraeidae. Mycorrhizal hyphae have been found in the fecal pellets of enchytraeids from pine litter (Fig. 7.6,

Ponge, 1991). Enchytraeids probably consume and further process larger fecal pellets and castings of soil macrofauna, such as collembolans and earthworms (Zachariae, 1964; Rusek, 1985). Thus it is clear that fecal contributions to soil by soil-dwelling invertebrates provide feedback mechanisms affecting the abundance and diversity of other soil-dwelling animals.

Enchytraeid densities range from $<$1000 individuals $m^{-2}$, in intensively cultivated agricultural soil in Japan, to $>$140,000 individuals $m^{-2}$ in a peat moor in the United Kingdom. In a subtropical climate, enchytraeid densities of 4000 to 14,000 individuals $m^{-2}$ occur in agricultural plots in the Piedmont of Georgia, USA, whereas higher densities (20,000 to 30,000 individuals $m^{-2}$) are found in surface layers of deciduous forest soils in the southern Appalachian mountains of North Carolina (van Vliet *et al.*, 1995). Although enchytraeid densities are typically highest in acid soils with high organic content, Didden (1995) found no statistical relationship over a broad range of data between average enchytraeid density and several environmental variables such as annual precipitation, annual temperature, or soil pH. It appears that local variability may be at least as great as variation on a wider scale, as enchytraeid densities show both spatial and seasonal variations. Vertical distributions of enchytraeids in soil are related to organic matter horizons. Up to 90% of populations may occur in the upper layers in forest and no-tillage agricultural soils, but densities may be higher in the Ah horizon of grasslands (Davidson *et al.*, 2002). Seasonal trends in enchytraeid population densities appear to be associated with moisture and temperature regimes (van Vliet, 2000).

Enchytraeids have been shown to have significant effects on soil organic matter dynamics and on soil physical structure. Litter decomposition and nutrient mineralization are influenced primarily by interactions with soil microbial communities. Enchytraeid feeding on fungi and bacteria can increase microbial metabolic activity and turnover, accelerate the release of nutrients from microbial biomass, and change species composition of the microbial community through selective grazing. However, Wolters (1988) found that enchytraeids decreased mineralization rates by reducing microbial populations and possibly by occluding organic substrates in their feces. The influence of enchytraeids on soil organic matter dynamics is therefore the net result of both enhancement and inhibition of microbial activity depending on soil texture and population densities of the animals (Wolters, 1988; van Vliet, 2000).

Enchytraeids affect soil structure by producing fecal pellets, which, depending on the animal size distribution, may enhance aggregate stability in the 600- to 1000-μm aggregate size fraction. In forest floors, these pellets are composed mainly of fine humus particles, but in mineral soils, organic matter and mineral particles may be mixed into fecal pellets with a loamy texture. Davidson *et al.* (2002) estimated that enchytraeid fecal pellets constituted nearly 30% of the volume of the Ah horizon in a Scottish grassland soil (Fig. 7.7). Encapsulation or occlusion of organic matter into these structures may reduce decomposition rates. Burrowing activities of enchytraeids have not been well studied, but there is evidence that soil porosity and pore continuity can increase in proportion to enchytraeid body size (Rusek, 1985; Didden, 1990).

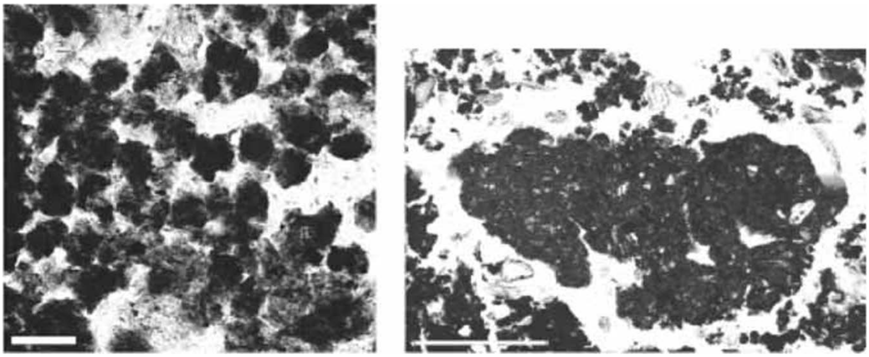



FIGURE 7.7 Thin-section micrographs of fecal pellets in a grassland soil. (a) Derived from enchytraeids (scale bar, 0.5 mm). (b) Derived from earthworms (scale bar, 1.0 mm) (from Davidson *et al.*, 2002).

Enchytraeids are typically sampled in the field using cylindrical soil cores of 5- to 7.5-cm diameter. Large numbers of replicates may be needed for a sufficient sampling due to the clustered distribution of enchytraeid populations (van Vliet, 2000). Extractions are often done with a wet-funnel technique, similar to the Baermann funnel extraction used for nematodes. In this case, soil cores are submerged in water on the funnel and exposed for several hours to a heat and light source from above; enchytraeids move downward and are collected in the water below. Van Vliet (2000) provides a comparison of modifications of this technique.

## MACROFAUNA

### MACROARTHROPODS

Larger insects, spiders, myriapods, and others are considered together under the appellation "macroarthropods." Typical body lengths range from about 10 mm to as much as 15 cm for centipedes (Shelley, 2002). The group includes a mixture of various arthropod classes, orders, and families. Like the microarthropods, the macroarthropods are defined more by the methods used to sample them rather than by measurements of body size. Large soil cores (10-cm diameter or greater) may be appropriate for euedaphic (dwelling within the soil) species. Arthropods can be recovered from them using flotation techniques (Edwards, 1991). Hand sorting of soils and litter is more time consuming, but yields better estimates of population size. In rare instances, capture–mark–recapture methods have been used to estimate population sizes of selected macroarthropod species, but the assumptions for this procedure are violated more often than not (Southwood, 1978). Pitfall traps have been widely used to sample litter- and surface-dwelling macroarthropods. This method collects arthropods that fall into cups filled with

preservative. Absolute population estimates are difficult to obtain with pitfall traps but the method yields comparative estimates when used with caution.

Many of the macroarthropods are members of the group termed “cryptozoa,” a group consisting of animals that dwell beneath stones or logs, under bark, or in cracks and crevices. Cryptozoans typically emerge at night to forage, and some are attracted to artificial lights. The cryptozoa fauna is poorly defined but remains useful for identifying a group of invertebrate species with similar patterns of habitat utilization.

## IMPORTANCE OF THE MACROARTHROPODS

The macroarthropods are a significant component of soil ecosystems and their food webs. Macroarthropods differ from their smaller relatives in that they may have direct effects on soil structure. Termites and ants in particular are important movers of soil, depositing parts of lower strata on top of the litter layer (Fig. 7.8). Emerging nymphal stages of cicadas may be numerous enough to disturb soil structure. Larval stages of soil-dwelling scarabaeid beetles sometimes churn the soil in grasslands. These and other macroarthropods are part of the group that has been termed ecological engineers (Jones *et al.*, 1994). Some macroarthropods participate in both above- and belowground parts of terrestrial ecosystems. Many macroarthropods are transient or temporary soil residents and thus form a connection between food chains in the “green world” of foliage and the “brown world” of the soil. Caterpillars descending to the soil to pupate or migrating armyworm caterpillars are prey to ground-dwelling spiders and beetles. Macroarthropods may have a major influence on the microarthropod portion of belowground food webs. Collembola, among other microarthropods, are important food items for spiders, especially immature stadia, thus providing a macro- to microconnection. Other macroarthropods, such as cicadas, emerging from soil may serve as prey for some vertebrate animals (Lloyd and Dybas, 1966), thus providing a link to the larger megafauna. Among the macroarthropods, there are many litter-feeding species, such as the millipedes, that are important consumers of leaf, grass, and wood litter. These arthropods have major influences on the decomposition process, thereby impacting rates of nutrient cycling in soil systems. The decomposition of vertebrate carrion is largely accomplished through the actions of soil-dwelling insects (Payne, 1965).

## OLIGOCHAETA (EARTHWORMS)

Earthworms are the most familiar and, with respect to soil processes, often the most important of the soil fauna. The importance of earthworms arises from their influence on soil structure (e.g., aggregate or crumb formation, soil pore formation) and on the breakdown of organic matter applied to soil (e.g., fragmentation, burial and mixing of plant residues). The modern era of earthworm research began with Darwin’s (1881) book, “The Formation of Vegetable Mould through the

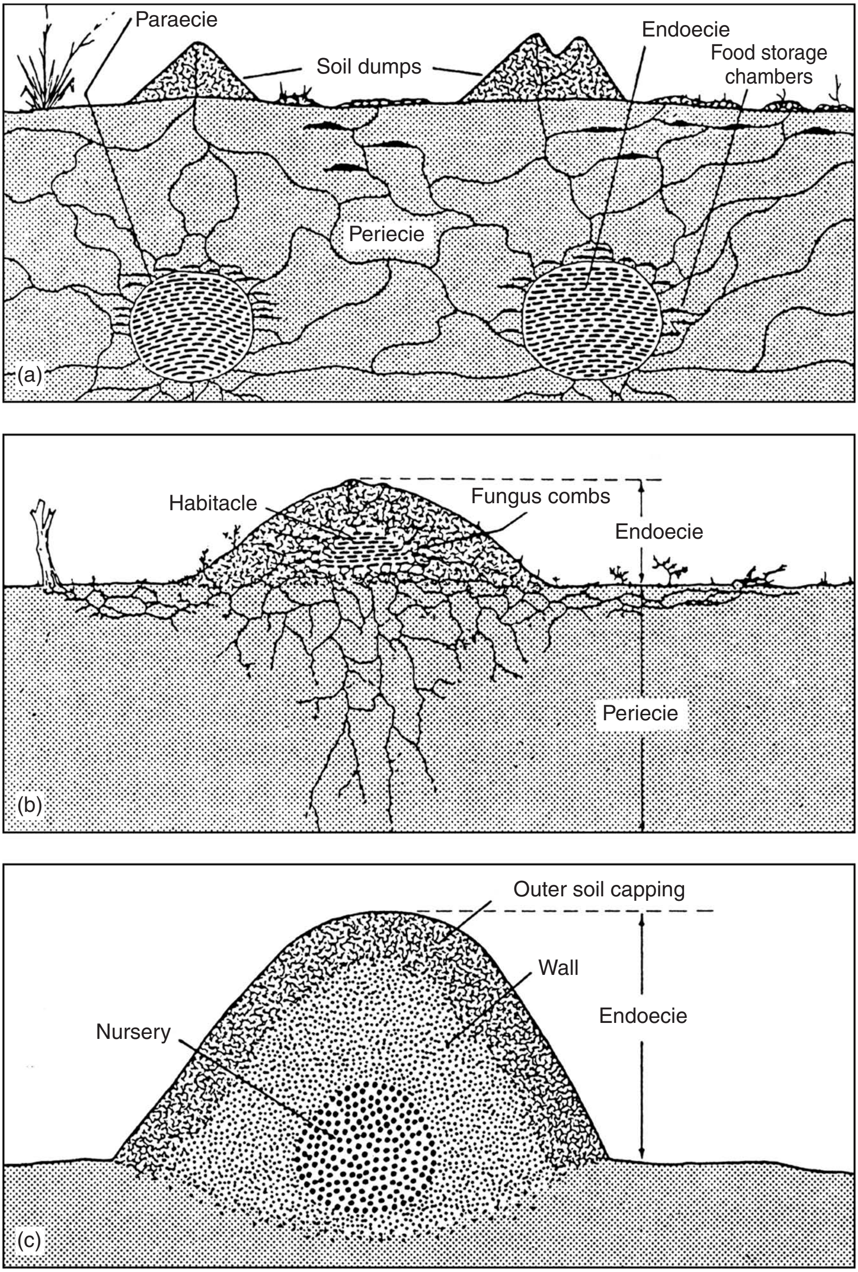



FIGURE 7.8 Termite mounds. Diagrammatic representation of different types of concentrated nest systems. (a) *Hodotermes mossambicu.* (b) *Macrotermes subhyalinus.* (c) *Nasutitermes exitiosus* (from Lee and Wood, 1971).

Actions of Worms, with Observations of Their Habits," which called attention to the beneficial effects of earthworms. Since then, a vast literature has established the importance of earthworms as biological agents in soil formation, organic litter decomposition, and redistribution of organic matter in the soil (Hendrix, 1995; Edwards, 1998).

Earthworms are classified within the Phylum Annelida, Class Oligochaeta. Species within the Families Lumbricidae and Megascolecidae are ecologically the most important in North America, Europe, Australia, and Asia. Some of these species have been introduced worldwide by human activities and now dominate the earthworm fauna in many temperate areas. Any given locality may be inhabited by all native species, all exotic species, a combination of native and exotic species, or no earthworms at all. Relative abundance and species composition of local fauna depend greatly on soil, climate, vegetation, topography, land use history, and, especially, past invasions by exotic species.

### Earthworm Distribution and Abundance

Earthworms occur worldwide in habitats where soil water and temperature are favorable for at least part of the year. They are most abundant in forests and grasslands of temperate and tropical regions and least so in arid and frigid environments, such as deserts, tundra, or polar regions. Earthworm densities in a variety of habitats worldwide range from $<10$ to $>2000$ individuals $m^{-2}$, the highest values occurring in fertilized pastures and the lowest in acid or arid soils (coniferous or sclerophyll forests). Typical densities from temperate deciduous or tropical forests and certain arable systems range from $<100$ to over 400 individuals $m^{-2}$, representing a range of from 4 to 16 g dry mass $m^{-2}$. Intensive land management (especially soil tillage and application of toxic chemicals such as common soil and plant pesticides) often reduces the density of earthworms or may completely eliminate them. Conversely, degraded soils converted to conservation management often show increased earthworm densities after a suitable period of time (Curry *et al.*, 1995; Edwards and Bohlen, 1996).

### Biology and Ecology

Earthworms are often grouped into functional categories based on their morphology, behavior, and feeding ecology and their microhabitats within the soil (Lee, 1985; Lavelle, 1983). Epigeic and epi-endogeic species are often polyhumic, meaning they prefer organically enriched substrates and utilize plant litter on the soil surface and C-rich upper layers of mineral soil. Polyhumic endogeic species inhabit mineral soil with high organic matter content ($>3\%$), such as the rhizosphere, while meso- and oligohumic endogeic species inhabit soil with moderate (1–3%) and low ($<1\%$) organic matter contents, respectively. Anecic species exploit both the surface litter as a source of food and the mineral soil as a refuge. The familiar *Lumbricus terrestris* is an example of an anecic species, constructing burrows and pulling leaf litter down into them. The American log worm (*Bimastos parvus*) exploits leaf litter and decaying logs with little involvement in the soil, making it an epigeic species. Epigeic species promote the breakdown and mineralization of surface litter,

whereas anecic species incorporate organic matter deeper into the soil profile and facilitate aeration and water infiltration through their formation of burrows.

### Influence on Soil Processes

Earthworms, as ecosystem engineers (Lavelle *et al.*, 1998), have pronounced effects on soil structure as a consequence of their burrowing activities as well as their ingestion of soil and production of castings (Lavelle and Spain, 2001; van Vliet and Hendrix, 2003). Casts are produced after earthworms ingest mineral soil or particulate organic matter, mix and enrich them with organic secretions in the gut, and then deposit the material as a slurry lining their burrows or as discrete fecal pellets. Excretion of fecal pellets can occur within or upon the soil, depending on earthworm species. Turnover rates of soil through earthworm casting range from 40–70 t $ha^{-1}$ $y^{-1}$ in temperate grasslands (Bouché, 1983) to 500–1000 t $ha^{-1}$ $y^{-1}$ in tropical savannas (Lavelle *et al.*, 1992).

While in the earthworm gut, casts are colonized by microbes that begin to break down soil organic matter. As casts are deposited in the soil, microbial colonization and activity continue until readily decomposable compounds are depleted. Mechanisms of cast stabilization include organic bonding of particles by polymers secreted by earthworms and microbes, mechanical stabilization by plant fibers and fungal hyphae, and stabilization due to wetting and drying cycles and age-hardening effects (Tomlin *et al.*, 1995). Mineralization of organic matter in earthworm casts and burrow linings produces zones of nutrient enrichment compared to bulk soil. These zones are referred to as the "drilosphere" and are often sites of enhanced activity of plant roots and other soil biota (Lavelle *et al.*, 1998). Plant growth-promoting substances have also been suggested as constituents of earthworm casts. Many earthworm castings from commercial vermicomposting operations are sold commercially as soil amendments to improve soil physical properties and enhance plant growth (Edwards, 1998).

Earthworm burrowing in soil creates macropores of various sizes, depths, and orientations, depending on species and soil type. Burrows range from about 1 to $>10$ mm in diameter and constitute among the largest of soil pores. Continuous macropores resulting from earthworm burrowing may enhance water infiltration by functioning as by-pass flow pathways through soils. These pores may or may not be important in solute transport, depending on soil water content, the nature of the solute, and chemical exchange properties of the burrow linings (Edwards and Shipitalo, 1998).

### Earthworm Effects on Ecosystems

Despite the many beneficial effects of earthworms on soil processes, some aspects of earthworm activities may be undesirable (Lavelle *et al.*, 1998, Parmelee *et al.*, 1998). Detrimental effects include: (1) removing and burying of surface residues that would otherwise protect soil surfaces from erosion; (2) producing fresh casts that increase erosion and surface sealing; (3) increasing compaction of surface soils by decreasing soil organic matter, particularly for some tropical

species; (4) riddling irrigation ditches, making them leaky; (5) increasing losses of soil N through leaching and denitrification; and (6) increasing soil C loss through enhanced microbial respiration. Earthworms may transmit pathogens, either as passive carriers or as intermediate hosts, raising concerns that some earthworm species could be a vector for the spread of certain plant and animal diseases. The net result of positive and negative effects of earthworms, or any other soil biota, determines whether they have detrimental impacts on ecosystems (Lavelle *et al.*, 1998). An effect, such as mixing of O and A horizons, may be considered beneficial in one setting (e.g., urban gardens) and detrimental in another (e.g., native forests). Edwards (1998) provides a review of the potential benefits of earthworms in agriculture, waste management, and land remediation.

## FORMICIDAE (ANTS)

Formicidae, the ants, are probably the most significant family of soil insects, due to the very large influence they have on soil structure. Ants are numerous, diverse, and widely distributed from arctic to tropical ecosystems. Ant communities contain many species, even in desert areas (Whitford, 2000), and local species diversity is especially large in tropical areas. Populations of ants are equally numerous. About one-third of the animal biomass of the Amazonian rain forest is composed entirely of ants and termites, with each hectare containing in excess of 8 million ants and 1 million termites (Hölldobler and Wilson, 1990). Furthermore, ants are social insects, living in colonies with several castes.

Ants have a large impact on their ecosystems. They are major predators of small invertebrates. Their activities reduce the abundance of other predators such as spiders and carabid beetles (Wilson, 1987). Ants are ecosystem engineers, moving large volumes of soil, as much as earthworms do (Hölldobler and Wilson, 1990). Ant influences on soil structure are particularly important in deserts, where earthworm densities are low. Given the large diversity of ants, identification to species is problematic for any but the taxonomist skilled in ants. Wheeler and Wheeler (1990) offer keys to subfamilies and genera of the Nearctic ant fauna.

## TERMITIDAE (TERMITES)

Along with earthworms and ants, termites are the third major earth-moving group of invertebrates. Termites are social insects with a well-developed caste system. Through their ability to digest wood they have become economic pests of major importance in some regions of the world (Lee and Wood, 1971; Bignell and Eggleton, 2000). Termites are highly successful, constituting up to 75% of the insect biomass and 10% of all terrestrial animal biomass in the tropics (Wilson, 1992; Bignell, 2000). While termites are mainly tropical in distribution, they occur in temperate zones as well. Termites have been called the tropical analogs of earthworms, since they reach a large abundance in the tropics and process large amounts of litter. Termites in the primitive families, such as Kalotermitidae, possess

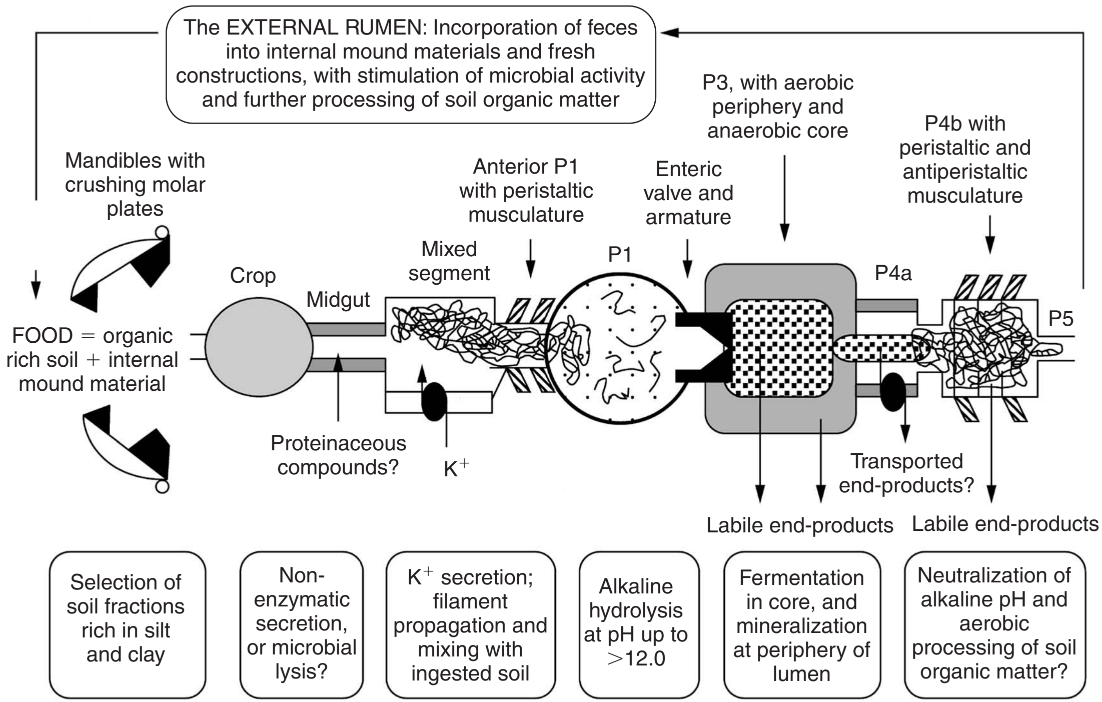



FIGURE 7.9 Hypothesis of gut organization and sequential processing in soil-feeding *Cubitermes*-clade termites. The model emphasizes the role of filamentous prokaryotes, the extremely high pH reached in the P1, and the existence of both aerobic and anaerobic zones within the hindgut. Major uncertainties have question marks. Not to scale (from Brauman *et al.*, 2000).

a gut flora of protozoans, which enables them to digest cellulose. Their normal food is wood that has come into contact with soil. Most species of termites construct runways of soil and some are builders of spectacular mounds. Members of the phylogenetically advanced family Termitidae do not have protozoan symbionts, but possess a formidable array of microbial symbionts (bacteria and fungi) that enable them to process and digest the humified organic matter in tropical soils (Breznak, 1984; Bignell, 1984; Pearce, 1997). A generalized sequence of events in a typical Termitinae soil-feeder gut is illustrated in Fig. 7.9 (Brauman *et al.*, 2000).

Three nutritional categories include wood-feeding species, plant- and humus-feeding species, and fungus growers. The last group lacks intestinal symbionts and depends upon cultured fungus for nutrition. Termites have an abundance of unique microbes living in their guts. One recent study of bacterial microbiota in the gut of the wood-feeding termite *Reticulitermes speratus* found 268 phylotypes of bacteria (16S rRNA genes, amplified by PCR), including 100 clostridial, 61 spirochaetal, and 31 Bacteroides-related phylotypes (Hongoh *et al.*, 2003). More than 90% of the phylotypes were found for the first time, but we do not know if they are active and participating in wood decay. Other phylotypes were monophyletic clusters with sequences recovered from the gut of other termite species. Cellulose digestion in termites, which was once considered to be solely due to the activities of fungi and protists and occasionally bacteria, has now been demonstrated to be endogenous to termites. Endogenous cellulose-degrading enzymes occur in the midguts of two species of higher termites in the genus *Nasutitermes* and in the *Macrotermitinae* (which cultivate basidiomycete fungi in elaborately constructed gardens) as well (Bignell, 2000).

In contrast to the C-degradation situation, only prokaryotes are capable of producing nitrogenase to fix $N_2$. This process occurs in the organic-matter rich, microaerophilic milieu of termite guts. Some termite genera have bacteria that fix relatively small amounts of N, but others, including *Mastotermes* and *Nasutitermes*, fix from 0.7 to $>21$ $\mu$g N $g^{-1}$ fresh wt $day^{-1}$. This equals 20–61 $\mu$g N per colony per day, which would double the N content if $N_2$ fixation was the sole source of N and the rate per termite remained constant (N content of termites assumed to be 11% on a dry weight basis) (Breznak, 2000). For an extensive exposition of the role of termites in the dynamics of soil organic matter and nutrient cycling in ecosystems worldwide, refer to Bignell and Eggleton (2000).

## SUMMARY

Soil fauna may be considered as very efficient means to assist microbes in colonizing and extending their reach into the horizons of soils worldwide. Their roles as colonizers, comminutors, and engineers within soils have been emphasized, but new technologies and global environmental issues are yielding new questions about how soil fauna contribute to the long-term sustainability of soils. The demand for taxonomic specialists for all groups of soil biota is increasing as we

currently recognize that molecular information alone is insufficient for many studies. Stable isotope technologies are revealing that some soil faunal species hitherto thought to be within one trophic group are not, thus leading to research about the structure and resilience of soil food webs. Information on the biogeography of soil fauna, their latitudinal gradient patterns, their relationship to aboveground hot spots and to land management strategies, as well as their taxonomic status and natural history, will be critical for understanding how microbes and soil fauna will interact and respond to multiple global changes (Wall *et al.*, 2001). For example, soil invertebrates can be invasive species, which, depending on the species, can affect soil carbon sequestration, soil fertility, and plant and animal health and result in economic and ecosystem change. For further reading on the roles of fauna in soil processes, see Coleman *et al.* (2004) and Wall (2004).

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PART

# III

# Concepts and Interactions

# 8

# The Ecology of Soil Organisms

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## INTRODUCTION

Ecology is the study of the interactions of organisms with one another and their environment. The name, Oecologie, provided by Haeckel in 1866, is based on the Greek term "oikos," the family household, which embodied the view of the environment and organisms as a household (Kingsland, 1991). Ecology was developed to provide a mechanistic backbone to the science of natural history that was popular but becoming somewhat passé in the late 1800s. There was great interest in providing a scientific basis for examining the mechanisms behind Darwin's concept of "survival of the fittest" and the process of natural selection. Because of this origin, the field of ecology is entwined with evolution and it is often presupposed that the basis of ecological relationships is one of long evolutionary history. Human disturbances to ecosystems can result in interactions among organisms that are not based on evolutionary history and provide opportunities to test this presupposition.

Ecology has recently moved from intellectual obscurity to prominence as a science that could provide tools for approaching environmental problems. Odum (1997) suggested that ecology has matured to "a basic science of the total environment." Contributions that ecology has made and will continue to make to science and society are a result of its interdisciplinary nature. Its roots are in geology,

chemistry, mathematics, and physics, as well as botany, zoology, microbiology, genetics, and molecular biology. The need to provide answers to ecological questions has driven methodological developments in multiple fields. Development of techniques for examining population and community structure at the molecular level has and will continue to provide answers to hypotheses that were initially proposed by Darwin.

As a field, ecology developed from research on plant and animal systems; microbial systems differ in significant ways. The "species concept" adopted by many plant and animal biologists defines a species as an interbreeding group of organisms that is reproductively (and genetically) isolated from other organisms. Most microbial species reproduce asexually. They do not interbreed and this part of the biological species concept does not apply. Genetic exchange is not a fundamental part of the bacterial life cycle and, for many species and environments, it is rare (Young, 1998). Yet, there are mechanisms that can cause genetic transfer between species, sometimes disparately related. This implies that bacterial species are not genetically isolated, so the second part of the biological species concept may also not apply.

Classification of organisms into species has two goals: (1) the classification should reflect the evolutionary history of the organisms and (2) it should be useful for dividing organisms into groups with different physiological and ecological characteristics. The biological species concept seems to satisfy both goals for plants and animals reasonably well (although it is now widely criticized). Before DNA could be manipulated in research, the species definition used by microbiologists differentiated individual species based on morphology or physiology, essentially adopting the second goal of taxonomy mentioned above as a species concept. Microbial taxonomy based on these definitions led to frustration. For much of the latter half of the 20th century, microbiologists abandoned the goal of having taxonomy reflect evolutionary history (Woese, 1994). Bacterial species are currently defined as a collection of strains with DNA–DNA reassociation similarity of $>70\%$. This creates groups that satisfy the goals of taxonomy, but in many cases it provides no evolutionary or phenotypic information about the groups created. It is no more universal than the biological species concept since it would not seem to apply to many well-recognized species of eukaryotes (for example, the human and chimpanzee DNA reassociation similarity is 98.4%) (Staley, 1997). With the sequencing of DNA in the past 20 years, the evolutionary basis for microbial taxonomy has returned. An individual gene sequence may provide narrow information about a potential phenotype or may provide a view of evolutionary relationships that is limited by the rate of mutation in the gene and the amount of horizontal transfer it has experienced. Taxonomy is currently moving toward genomic approaches (i.e., genome-wide sequencing efforts) that may allow species to be defined by inferring evolutionary relationships from large sets of gene sequences, while at the same time obtaining information about the presence of many genes with important phenotypic consequences.

The organization of the field of ecology reflects a hierarchical approach that includes studies of individuals, populations, communities, and ecosystems. Studies

that examine individuals focus on responses to and impacts on abiotic factors at evolutionary, physiological, and behavioral levels. Studies of populations include evaluation of the impacts of intraspecific competition on the density of organisms and social, genetic, and spatial organization of organisms. Community level work involves interspecific competition, changes in the number and diversity of species as organized by multiple species interactions, succession, and equilibrium and non-equilibrium dynamics of community regulation. The broadest subdivision of ecology is ecosystems ecology, which focuses on food webs, energy transformations, and nutrient transfers within systems and across global scales.

In this chapter, we focus on the processes that drive community structure (number and types of species) and the resultant impacts on ecosystem function (processes of energy transformations and nutrient turnover). An additional impetus for the emphasis on ecology in studying microorganisms and their roles within soils in the 21st century is the need for sustainable agriculture, forestry, and natural resource management. The integrative approach provided by the field of ecology aids in understanding the complexity of soil systems necessary to manage systems that will not become degraded over the long term. In return, examining soils as complex integrated systems has great potential to influence ecological concepts and the science of ecology in general, and soils can be described as the last ecological frontier.

## MECHANISMS THAT DRIVE COMMUNITY STRUCTURE

A large part of ecology is the study of how organisms become distributed in the environment. Why are these species found in one area and not in others? The area an organism lives in is called its "habitat." Organisms that live in one habitat make up the "community," and the numbers and kinds of organisms present are referred to as "community structure." The emerging field of community assembly theory is concerned with the search for rules governing community structure using a synthetic, holistic approach (Keddy, 1992; Weiher and Keddy, 1999). Communities are composed of populations or subpopulations of various species. A "population" is a collection of organisms belonging to a single species with potential for interaction. This makes the spatial scale of a population dependent on the mobility of the species. A study may encompass only part of a true population (e.g., migrating species) or encompass multiple, isolated populations (e.g., soil bacteria). Given the degree to which species are differentially mobile, it is normal for both situations to arise in the same study.

There are numerous examples of studies for which predicting the population density of organisms is necessary (e.g., for endangered species, commercial fish, timber). In soil microbiology, it is desirable to be able to forecast population dynamics of plant pathogens or inoculant species such as rhizobia, biocontrol agents, and genetically modified organisms. Population dynamics form the basis of community assembly, and interactions with the community have a profound influence

on populations. Identifying populations and studying them *in situ* are difficult and have to date limited true understanding of the complexity of community assembly in soil systems.

## PHYSIOLOGICAL LIMITS

Each species has a unique set of limitations on the conditions under which individuals, and therefore populations, can grow and reproduce. Shelford's Law of Tolerance states that there is a maximum and minimum value for each environmental factor, beyond which a given species cannot survive. This is usually discussed with respect to environmental characteristics known as "modulators," such as temperature, pH, or salinity. Modulators impact the physiology of organisms by altering the conformation of proteins and cell membranes and the thermodynamic and kinetic favorability of biochemical reactions (see Chap. 2). For each environmental modulator, species also have an optimal range, within which maximum population growth occurs. Tolerance to modulators can be interactive; for example, tolerance to temperature extremes may be broader at one pH than another. Normally the geographical range of a species coincides with areas where environmental conditions are within the optimal ranges for the species, with the most optimal conditions at the center of the geographical range. The effects of species being in habitats with modulators outside their tolerance levels are listed in Table 8.1, along with biochemical strategies used by microorganisms that exist under these "extreme" conditions.

Resources are physical components of the environment that are captured by organisms for their use, such as N, energy, territories, or nesting sites. Shelford's law can be applied to most resources, but the responses to different resources are highly interactive. This is partially captured in Liebig's Law of the Minimum, which states that the resource in lowest supply relative to organismal needs will limit growth. At very low levels of a resource, the organism is unable to accumulate the resource in adequate quantities for metabolism. At very high levels, resources can also be toxic or inhibit growth.

The response of a species to environmental conditions or resources depends on the genetic makeup of the species. The limits and optima are determined through natural selection and other mechanisms that affect the genome. All organisms have some degree of "phenotypic plasticity," or the ability to adapt to the environment. In microorganisms, a change in an environmental condition can induce expression of alternative phenotypes (e.g., proteins, phospholipids) that are adapted to the new conditions, broadening the range of conditions acceptable for the species. The cost associated with this ability is extra genetic material that must be duplicated with each cell division, resulting in lower efficiency of resource use. This strategy can be efficient in fluctuating environments such as the soil surface. At the other extreme, endosymbionts that live continuously within the host and depend on the homeostasis of their host can have reduced genomes (Silva *et al.*, 2001; Gil *et al.*, 2003).

TABLE 8.1 The Effects of Physical Stresses (Modulators) on Microorganisms and the Biochemical Adaptations They Induce (Modified from Paul and Clark, 1996)

| Modulator | Effects on cell | Biochemical adaptation | Organisms that have required adaptation |
|---|---|---|---|
| Temperature | Denaturation of enzyme; change in membrane fluidity | Production of proteases and ATP-dependent chaperones (Derré *et al.,* 1999); production of cold-tolerant enzymes by amino acid substitution (Lönn *et al.,* 2002); increases in intracellular trehalose and polyol concentrations and unsaturated membrane lipids, secretion of antifreeze proteins and enzymes active at low temperatures (Robinson, 2001) | Thermophiles, psychrophiles |
| Water deficit or salt stress | Dehydration and inhibition of enzyme activity | Changes in composition of polysaccharides produced (Coutinho *et al.,* 1999); maintaining salt in cytoplasm and uptake or synthesis of compatible solutes (Roeßler and Muller, 2001) | Osmophiles, xerophiles, halophiles |
| pH | Protein denaturation; enzyme inhibition | Increased intrasubunit stability in proteins afforded by increased hydrogen bonds and stronger salt bridges (Settembre *et al.,* 2004); organisms that can secrete a surplus of protons or block extracellular protons from the cytoplasm by blocking membrane composition (de Jonge *et al.,* 2003); stress regulator genes (de Vries *et al.,* 2001) | Acidophiles |
| Aeration stress | Oxygen radicals damage membrane lipids, proteins, and DNA | Detoxification of oxygen radicals by catalase and superoxide dismutase (Wu and Conrad, 2001) | Obligate anaerobes, methanogens, sulfur and N users |

## INTRASPECIFIC COMPETITION

Liebig's Law of the Minimum was developed for the nutrition of agricultural plants, but can also be applied to populations. Reproduction (birth of new organisms) and death are the fundamental processes regulating change in population size

over time. Low abundance of resources, or other nonideal environmental conditions, reduces the reproduction rate or increases the death rate in a population, in addition to lowering rates of growth or activity for individual organisms. Reproductive rates are more sensitive to changes in the environment than metabolic rates or death rates since an individual can often survive under conditions under which it cannot reproduce. If a population grows, resources decline and some individuals will not obtain resources in adequate supply. This affects individual reproduction. It is also an example of natural selection, since the individuals that are more successful at obtaining and using resources will make a greater contribution to the genetic makeup of the next generation. If the organisms within a population are members of the same species, this is "intraspecific competition."

For each species there is a gradient of habitat suitability that is determined by environmental conditions and resources. Ecologists need quantitative information on the suitability of habitats to predict population dynamics in other areas, in the future, and due to changes in community structure. The theoretical basis for these relationships was formalized by Pearl and Reed (1920), who promoted the logistic growth equation that had been previously described by P. F. Verhulst in 1838. The equation is an attempt to relate the specific growth rate of a population ($\mu$) to the environment. The probability of an individual reproducing ($b$) minus the probability of death ($d$) per unit time is equal to $\mu$ and is a direct manifestation of the suitability of the habitat. The differential equation for logistic growth is

$$\frac{\mathrm{d}N}{\mathrm{d}t} = \mu \bullet N$$

where

$$\mu = r \bullet \left(\frac{K - N}{K}\right)$$

and where $N$ is the number of individuals in the population, $t$ is time, and $\mathrm{d}N/\mathrm{d}t$ is the change in $N$ over time. The intrinsic growth rate of the population ($r$) is the value $\mu$ approaches when resources are not limiting growth and there is no intraspecific competition. The effects of environmental conditions other than resources, for instance temperature, are modeled by changes in $r$. The number of individuals that the resources of a habitat can support ($K$) is referred to as the carrying capacity, and models intraspecific competition with a constant level of resource supply. Other impacts of the population on the environment, such as the accumulation of waste, are also modeled by $K$. As a population grows, the resources must be shared among many individuals, decreasing the reproduction rate and increasing the rate of death. In the equation, $N$ approaches $K$, causing $\mu$ to approach 0. If the population is above the carrying capacity, it cannot be supported by the resources present, $\mu$ becomes negative, and the population declines. The relationship in which population growth rate is sensitive to population size is known as density-dependent population regulation.

The logistic growth equation is used by population ecologists mainly as a conceptual tool, rather than to forecast changes in population size, because the mechanisms of population regulation are not explicitly modeled in terms of reproduction and death rates. It is also rare in plant and animal populations for individuals of different ages to have the same rate of death or equal probabilities of reproduction. Intraspecific competition often affects certain classes of individuals more than others. The common method of forecasting population sizes is currently the use of structured demographic models that consider the effects of age, size, or developmental stage on probabilities of reproduction and death. Populations of each class of organism and the transition of individuals between classes are tracked separately in empirical models, using matrix algebraic methods. While the assumptions of the logistic growth equation are thought to be more appropriate for microorganisms such as bacteria and yeasts, structured demographic models have also been used for these organisms to take into account a dormant stage or vegetative (nonreproductive) stage.

Lifetime patterns of growth and reproduction, including timing of reproductive and dormant stages are known as a species' "life history." Life history strategies have important consequences for population dynamics. The logistic growth equation led to MacArthur and Wilson's (1967) model of *r* and *K* selection, which, although controversial, is still used as a method of generalizing about species life histories. *K*-selected species (with high *K* values and low *r* values) are selected for traits that favor the persistence of individuals under conditions of scarce resources and high intraspecific competition. These conditions occur when populations remain near their carrying capacity (*K*). In contrast, *r*-selected species have the opposite characteristics, with relatively high efficiency in converting resources to offspring. The *K*-selected strategy is an adaptation to environments in which conditions are relatively stable, resulting in density-dependent mechanisms of population regulation, while the *r*-selected strategy is an adaptation to a variable environment with high levels of resources. Hence, environments can be classified as *r*- or *K*-selecting.

Pianka (1970) made several other predictions about the correlates of *r/K* selection. An *r*-selected species was predicted to have more variable population size (typically below carrying capacity), weak intra- and interspecific competitive interactions, rapid maturation, early reproduction, small body size, semelparity (one reproductive event per lifetime), short life span, and high productivity. Ecologists soon realized that selection pressure and reproductive value at different ages can lead to traits opposite to those predicted by the correlates of the *r/K*-selection model (Reznick *et al.*, 2002). Like the logistic growth equation, the *r/K*-selection model is now used mainly as a conceptual tool to explain life history and has been supplanted by structured demographic models that can be used to test specific hypotheses about life history evolution.

The classification of organisms as *r*- or *K*-selected is common in soil microbiology. Typically, colonies are classified based on the amount of time it takes for the colony to appear in laboratory isolation medium. These designations must be made with reference to a particular environment. Laboratory isolation conditions

represent a small range of the conditions encountered in the environment. The environment of a batch culture changes continuously as nutrients are not replenished and wastes are not removed. Hence *r*-selected species can be positively identified (with respect to the isolation conditions), but *K*-selected species cannot. Soil microbiologists have created other classifications similar to the *r/K*-selection dichotomy, but are focused more on the species' preferred resources than on intraspecific competition. In 1925, S. Winogradsky used the term autochthonous to describe organisms that grow steadily on organic matter with a constant presence in the environment and zymogenous for organisms that proliferate on fresh organic matter (Panikov, 1995). In another scheme, oligotrophs grow only at low nutrient levels, while copiotrophs grow quickly at high nutrient levels.

An early model that linked population growth and environmental resources was proposed by Jacques Monod in 1957 (Panikov, 1995). The Monod model was developed for microbial growth in chemostats, but is now used in a wide array of situations for macro- as well as microorganisms. It has been modified to account for a variety of situations, such as colimitation by multiple resources, growth inhibition, and migration. Maintaining the same notation as above, one version is

$$\frac{\mathrm{d}N}{\mathrm{d}t} = \mu \bullet N$$

where

$$\mu = \mu_0 \bullet \left( \frac{R}{K_s + R} \right) - d$$

and

$$\frac{\mathrm{d}R}{\mathrm{d}t} = -\frac{\mu \bullet N}{Y} - m \bullet N.$$

The maximum reproduction rate under nonlimiting resources in this equation is $\mu_0$. The concentration of resources in the environment is given by $R$, and the change in $R$ over time is d$R$/d$t$. The half-saturation constant, or resource concentration at which the reproduction rate is at half its maximum, is $K_s$. Maintenance utilization of resources, $m$, is the amount of resources used by organisms to stay alive (not reproduce). The growth yield, $Y$, is the amount of biomass produced per unit of resource consumed and accounts for extra utilization of resources during reproduction that are not retained in biomass. Intraspecific competition is explicitly modeled through the impacts of population size and growth rate on $R$, which then influences future population growth. The number of variables required for this model is double that of the logistic equation, but the simultaneous modeling of population growth and environmental resources extends the utility of the model. Additional terms added to the third equation can describe inputs of resources such as through diffusion, physical movement, or primary production, as well as losses such as leaching.

## DISPERSAL IN SPACE AND TIME

Another layer of realism is added to models of population dynamics by considering the spatial processes of organismal movement (immigration, emigration). Species are often distributed in space in multiple populations that are linked by migration. The first attempt to deal with this situation by Levins (1969) ignored population dynamics within each habitat patch, assuming that each patch was of the same quality and had the same probabilities of local population extinction and emigration. The scenario described is the definition of a “metapopulation.” Migration creates significant feedback between populations, resulting in persistence of a species whenever migration rate is greater than population extinction rate.

Populations of species that are not in decline often correspond with a different spatial distribution called a source-sink metapopulation (Harrison, 1991). In this distribution, there is a large habitat with a thriving population in no danger of local extinction and other smaller populations in less suitable habitats. Since emigration is often density-dependent, the thriving population regularly produces emigrants while the other populations do not. Hence, the less suitable habitats are kept populated, over the long term, by emigration from the large “source” population(s). These spatially distributed patterns for species populations have been shown to play an important role in the global population dynamics of species and are now a fundamental part of population viability analysis in conservation ecology.

Active dispersal involves the expenditure of energy by the organism. Passive dispersal occurs due to the movement of material the organism is attached to or caught in (e.g., wind or water). Passive dispersal can be truly passive with no energy expended, or an organism may prepare morphologically and physiologically for passive dispersal by entering a new life stage. Stages for dispersal are typically more resistant, dormant, or mobile than growth stages. The fruiting bodies and spores of fungi and myxococci are examples of elaborate life stages for passive dispersal. Passive dispersal of bacteria with water flow can occur if the bacteria are not adsorbed onto immobile soil particles or protected by soil structure. Fecal coliforms applied to the soil surface with manure can move several meters and contaminate ground or surface waters in some situations, particularly when preferential flow paths limit interaction of the water and cells with the soil. Cell size limits the passive movement of organisms with water in soil due to the sieving effect of soil particles. Larger-celled microbes such as yeasts and protozoans do not experience passive dispersal to as great a degree as bacteria and viruses; however, nondormant protozoans are typically engaged in active dispersal to obtain food. Plant roots, seeds, fungal spores, and chemical substrates found within several centimeters of particular soil bacteria have been shown to induce chemotactic responses (active dispersal) although the extent to which this occurs naturally is unclear (Murphy and Tate, 1996).

Passive dispersal of hyphal fungi is normally restricted to spores. Spores may be produced in the soil, such as by arbuscular mycorrhizal fungi, or in sporocarps (fruiting bodies) above the soil surface. Sporulation is often induced by environmental cues such as moisture. Spores are also dispersed by animal activity both above and below ground. Vegetative growth of fungal hyphae can also be considered

a form of active dispersal since new areas are being explored. Fungi often have distinct forms of hyphal growth for nutrient acquisition versus dispersal. Hyphae for dispersal, such as rhizomorphs, grow more rapidly, are thicker and tougher, and may be formed by anastomosis (cellular fusion) of multiple smaller hyphae (Rayner *et al.*, 1999). The strategy is to invest little and maintain impermeable surfaces during exploration of a resource-poor environment until a resource-rich patch is encountered. Upon encountering such a patch, there is a proliferation of thinner, more permeable hyphae with a higher surface-to-volume ratio.

The ability of an organism to enter a dormant phase can also be seen as a dispersal mechanism, but through time rather than space. This form of dispersal is one version of the "storage effect," by which reproductive potential is stored across time, resulting in higher reproduction rates under favorable environmental conditions. Entrance of individuals into a dormant life stage may be a developmentally programmed event for some species or may be induced to avoid density-dependent competition. For many organisms, life stages that facilitate passive dispersal in space are also optimal for dispersal in time. This is true of plant seeds and fungal spores. A general state of dormancy for soil microorganisms is indicated by the increase in numbers and metabolic activity when soil is amended with water or nutrients. Bacterial cells entering a dormant stage are known to undergo a suite of biochemical and morphological changes, including reduction in size. "Dwarf" cells ($<0.07\,\mu m^3$ biovolume or $<0.3\,\mu m$ diameter) make up the majority of bacterial cells in soil (Kieft, 2000).

## PREDICTING POPULATION GROWTH

The ideas presented in the previous sections have depicted population growth in terms of deterministic models because these are the most convenient tools we have for conceptualizing and exploring biological mechanisms. Population growth in nature is not so neatly defined. Even deterministic models can have unpredictable behavior and extreme sensitivity to initial conditions, leading to "chaotic dynamics" (Hastings *et al.,* 1993). Stochastic processes and other processes that are not density-dependent can have profound impacts on populations. Examples of density-independent factors include weather, physical disturbance (e.g., avalanche, tillage), or the application of broad-spectrum toxins (e.g., antibiotics, insecticides).

The term "population regulation" has been equated to "a long-term stationary probability distribution of population density," and it is now recognized that such population regulation is necessarily density-dependent (Turchin, 1995). Over the long term, the absence of population regulation leads to either extinction or infinite population growth. Hence, the majority of populations display density-dependent patterns when tested by experimental manipulations or time-series analysis.

## INTERSPECIFIC COMPETITION

We have seen in the previous sections how a species' geographic range and occupation of particular habitats is limited by the species' adaptations to environmental

conditions, resource levels, and life-history traits. The effects of abiotic factors on survival or growth rate of a population could be plotted with each axis corresponding to one factor. If we imagine many axes, each defining one dimension of an *n*-dimensional space, the region of this space suitable for growth of a species is what Hutchinson (1957) envisioned as the species' "fundamental niche." The fundamental niche of a species is all combinations of environmental conditions that are acceptable for the persistence of a population. Because of predation and competition with other species, populations of a species will not be present at all habitats that satisfy the species' fundamental niche. The reduced hypervolume corresponding to the conditions that a species is actually able to occupy is called its "realized niche." Interactions between species are fundamental processes in defining which species will be present in a given location.

Interspecific competition can operate according to the same mechanism as intraspecific competition, except that the individuals competing are from different species. A finite pool of resources is available at any given time, and if resources are consumed faster than they are replenished, growth rates decline. The strongest competitors are able to maintain higher growth rates despite lower levels of resources, and they drive resources yet lower. This form of interspecific competition is known as "resource-based competition" or "exploitative competition." Tilman (1982) suggested that if resources are replenished at a constant rate in a stable environment, the population of each species would reach an equilibrium point at which death rates equal reproduction rates. Hence, $\mu$ is equal to 0 at this equilibrium population density. According to the Monod model above, the equilibrium resource concentration ($R^*$) at the equilibrium population density for a given species is

$$R^* = \frac{d \bullet K_s}{\mu_0 - d}.$$

At resource concentrations below $R^*$, a population's reproduction rate is lower than its death rate. When multiple species use the same pool of resources, all populations will attempt to grow to their equilibrium levels. The species with the lowest value for $R^*$ will determine the equilibrium resource concentration, resulting in the local elimination of other species because of resource levels too low to support them.

Given this description of interspecific competition, how do similar species coexist? One key component of the resource-based model is the presence of constant resource supply rates in the environment. Spatial and temporal variability in resources leads to growth being limited by different resources in different times and places. Tilman (1982) suggested that the number of similar species that can coexist in a habitat should be equal to the number of potentially limiting resources used in that habitat, since species that are superior competitors for one resource are typically not as competitive for others. Another important assumption of the model is constancy of death rates. Discussion of density-independent mortality

factors has suggested that this is a departure from reality. Species that are *r*-selected may be outcompeted under normal conditions, but flourish when mortality spikes for the dominant species. "Fugitive species" avoid competition by dispersing into habitat patches where the dominant species has become locally extinct. Mortality rates can also be altered through "interference competition," in which one competing species impacts another through direct aggressive action rather than resource use. Examples include human efforts to control agricultural pests and physical attacks between animals. In soil, the degree of competition that is mediated by interference mechanisms is unknown, but there is the potential for interference competition to play an important role. This type of competition would be likely in systems containing antibiotic-producing organisms. Davelos *et al.* (2004) confirmed Waksman's much earlier observations when they found a wide variety of antibiotic production and resistant phenotypes present in soil streptomycetes at one location, suggesting many organisms capable of competitive interference. It also suggests that organisms have developed mechanisms to avoid this type of interference.

Similar species evolve to use different subtypes of the same resource, or their niches can shift in other ways. This is known as resource partitioning and was taken as some of the first evidence of competition and natural selection. Considering the mechanisms of species coexistence to be components of the niche, we arrive at the conclusion of Hanski *et al.* (1995) that the niche is equivalent to an ecological species concept. The concept that no two species having identical niches can coexist is the "competitive exclusion principle." So, how similar can two species be and still coexist? This has been explored theoretically to a limited extent (beginning with MacArthur and Levins, 1967). In reality, extinctions due to competition have been documented, but evolution can allow an environment to be partitioned into an astonishing array of niches. For example, Rozen and Lenski (2000) showed that a pure culture of *Escherichia coli* developed spontaneously into distinct subtypes that coexisted because of physiological (niche) differences. Niches can shift in terms of environmental tolerances as well, resulting essentially in species living in different habitats.

In soil, competition has been exploited as a mechanism for biocontrol, but it has also been blamed for the failure of many soil inoculation programs. Fluorescent pseudomonads have been shown to suppress a variety of plant pathogens by secretion of antibiotics (interference competition) and siderophores, which sequester iron (resource competition). Strains of *Fusarium oxysporum* that are nonpathogenic can be superior competitors for carbon and root colonization sites (Alabouvette *et al.*, 1996). Organisms introduced into sterilized soil often survive, while populations decline rapidly in nonsterile soil. The relatively short half-life of introduced populations has been observed for a variety of groups, including some biocontrol agents, rhizobia, fecal organisms, and genetically modified microbes. This has been attributed to competition, but could also be a result of trophic interactions, the type of biological interaction described next. In rare cases, inoculated populations have survived (in reduced numbers compared to the inoculum size) if the

environment is modified to match their niche requirements or they are naturally strong competitors.

## DIRECT EFFECTS OF EXPLOITATION

Trophic interactions refer to the transfer of energy or nutrients from one organism to another. The stored energy or nutrients of the prey serve as a resource for the consumer. Evolution has invented an array of methods for consumers to steal stored resources. In studying energy flow through an ecosystem, it may be useful to categorize consumers into trophic levels (e.g., herbivores and carnivores). To understand the impact of exploitation on population dynamics it is important to know if the consumer is a predator, causing the immediate death of prey so that prey resources can be consumed in a single event, or is a parasite, obtaining only a portion of the prey's resources without killing it, so that the same organism can be used in the future. Another important distinction is between a generalist, who consumes many different prey species, and a specialist, which consumes very few. As with all neat categories in ecology, there is actually a gradient of lifestyles that fall between the extremes.

Exploitation in soil biota is widespread. Most soil animals, including protozoa, nematodes, collembola, mites, earthworms, etc., obtain their resources through exploitation of bacteria, fungi, or plant roots. Invertebrates that ingest plant detritus normally get most of their energy and nutrients from microorganisms residing on the detritus (their "prey") rather than directly from the detritus. Many species of fungi have been shown to attack bacterial colonies and other fungi, and there are also fungi that attack soil animals. *Bdellovibrio* is a bacterial predator that attacks other bacteria. All organisms also appear to serve as a habitat for an assemblage of smaller organisms, many of which are parasitic.

A predator's effect on prey population dynamics is to increase the death rate. This effect can be modeled, along with predator population dynamics, using the Lotka–Volterra equations shown here (adapted to the notation used in previous sections):

$$\frac{\mathrm{d}N}{\mathrm{d}t} = \mu \bullet N$$

where

$$\mu_\mathrm{R} = b_\mathrm{R} - a \bullet N_\mathrm{C},$$

and

$$\mu_\mathrm{C} = e \bullet a \bullet N_\mathrm{R} - d_\mathrm{C}.$$

The subscripts R and C denote properties of the prey and predator populations, respectively. Death rate of the prey is a function of predator population density, and reproduction rate of the predator is a function of prey population density. The

number of attacks per unit time per predator, *a,* is assumed to be constant, but the total number of prey killed per unit time (calculated $aN_CN_R$) increases due to either increased prey or predator abundance. Predator reproduction rate is proportional to the number of prey killed, but also depends on *e*, the efficiency with which the predator can use prey to reproduce. Values of *e* less than 1 imply that a single predator must kill multiple prey organisms to reproduce, while values greater than 1 imply that the predator can reproduce using the resources from a single prey organism.

The attack rate of predators on prey is normally constant over only a limited range of prey and predator population densities. The total number of attacks per unit time per predator ($aN_R$) will reach an asymptote as prey population continues to rise, because of predator satiation, the minimum handling time required for each kill, and time spent performing other activities. Attack rates may also decline when population density of a particular prey species is below a threshold level, if the predator does not invest energy in pursuing prey that is too scarce. These phenomena can be incorporated into the Lotka–Volterra model using a nonlinear "functional response" in place of *a.*

Predators will aggregate in patches of high prey density. At very high predator population densities, attack rates may decline because other resources become limiting. Predators will then disperse into less resource-rich, but also less competitive, habitats. This is a consequence of intraspecific competition. Predatory pressure is also a factor in habitat quality for the prey. Predator-free patches serve as refuges for the prey population and can significantly impact metapopulation dynamics. Elliott *et al.* (1980) found that a finer-textured soil contained more bacteria protected from predation by nematodes. The finer-textured soil contained a larger proportion of pores too small for the nematodes to utilize. Amoebae were able to use these pores, and there was a greater increase in growth of nematodes that preyed on both bacteria and nematodes when amoebae were added to the fine- compared to a coarse-textured soil.

Parasitism is a considerably more complicated phenomenon to model than predation because prey are weakened by parasites, which impacts reproduction and death rates. Parasitism can decrease the accumulation of biomass or rate of development. In terms of the Monod model discussed above, parasitism may cause the infected subpopulation to have a decreased maximum growth rate ($\mu_0$) or to waste resources through increased maintenance utilization (*m*). Parasitism also typically increases the death rate, either through prolonged exposure to the parasite or by making the prey more sensitive to other causes of mortality.

The details of the route of transmission of a parasite between hosts are critical to understanding how the parasite is spread. Some parasites are able to colonize new hosts from dead tissue. For example, the plant root pathogens in the genera *Gaeumannomyces*, *Rhizoctonia*, and *Pythium* are able to live saprophytically within plant residue and colonize new roots from these habitats. Higher quality habitat patches allow pathogenic hyphae to grow farther through the soil (to at least 15 cm) to colonize new roots. The probability distribution of colonization of a root from a particular inoculum source would also depend on a variety of other

factors such as the species involved, temperature, moisture, and soil texture. Planting crops at wider distances apart (i.e., reducing host density) is known to reduce the spread of root diseases because the practice limits dispersal. Some parasites are transferred by other species or other components of the environment (vectors), and their spread is tightly linked to dynamics of these factors. In soil, fungal spores, bacteria, and viruses are transported passively in water and can be transferred by invertebrate vectors such as plant-parasitic nematodes and mites.

## INDIRECT EFFECTS OF EXPLOITATION

Unlike a nonliving resource, the genetic makeup of prey species will respond to exploitation through evolution, resulting in defensive adaptations. Defenses from exploitation can take a variety of forms, including behavioral, morphological, or biochemical defenses. Evolution can also result in the development of new attack strategies in consumers, resulting in a continual coevolutionary arms race between consumers and their prey. Exploitation pressure can be regulated by exploitation at higher trophic levels in a process called a trophic cascade. For example, if carnivores limit the population size of herbivores through heavy predation, then the pressure on plants from herbivory will be low. Consider in the equation above how an increase in death rate ($d$) for herbivores would affect the equilibrium concentration of the herbivore's resource ($R^*$). The herbivore's resource is of course the plant population density. In this case, competitive interactions will be strong for plants and carnivores, but relatively weak for herbivores where response to predation will be important. A trophic cascade is often the basis of strategies in biocontrol. For example, *Trichoderma herzianum*, a mycoparasitic fungus that attacks the root pathogen *Rhizoctonia solani*, has been introduced to control *Rhizoctonia* density.

Exploitation can have a large influence on the outcome of competitive interactions between prey species. Exploitation can contribute to the coexistence of competing prey species by reducing the population size of the superior competitor. This results in increased resource abundance and ameliorates competition. Paine (1969) called predators that perform this function "keystone species." The term has since been used to describe any species whose effects on an ecosystem are more than would be expected based on their biomass in a given system. There is a trade-off between competitive ability and anti-predator adaptations that allows keystone predation to take place. Ability to avoid exploitation is an alternative niche dimension that species can evolve to utilize.

When representative species are tested, most predatory soil fungi, protozoa, nematodes, and collembola will utilize multiple prey species. However, all also show feeding preferences for, or enhanced benefits from, particular prey species. It is therefore likely that predation regulates community composition (at the species level) by mediating competition between microbes or plant species. The former has been difficult to test because examining microbial community dynamics *in situ* is complicated; results from laboratory studies do not always provide adequate information about how natural systems operate. Klironomos and Kendrick

(1995) found that saprophytic fungi are often the preferred food source for arthropods, but arthropods will consume mycorrhizal fungi where saprophytic fungi are not available. These relationships can impact competition among plant species by altering the nutrients available from mycorrhizas to plants. Bever (2003) provides a comprehensive review of the mechanisms by which microbes mediate competition in plant communities.

In ecosystems, trophic relationships between organisms result in a complex web of interactions (a food web). A large number of organisms involved have multiple prey or multiple predators. Omnivorous predators utilize multiple prey from different trophic levels and may at times be in competition with potential prey. The study of how food web structure interacts with community composition and ecosystem processes is a field still in development. Experimental work has been performed largely with simple communities of protists and bacteria in microcosms. One preliminary conclusion from this work is that a greater number of trophic levels, or greater overall complexity, decreases the stability of constituent populations (Morin, 1999). However, this conclusion is in contrast to predictions of the constant connectance hypothesis, which is based on the observation that each species is less dependent on a single resource in more complex systems, providing more of a buffer to environmental fluctuations (Martinez and Dunne, 1998).

When microorganisms are included in soil food webs, the increase in complexity on the species level has been viewed as overwhelming. Microorganisms are normally represented by undifferentiated pools of biomass or are divided into very broad groups (e.g., fungi and bacteria). This is understandable because of the enormous diversity of soil microorganisms, the often unknown role of each taxon in a food web, and the fact that the focus of soil food web studies has typically been biogeochemical processes, not community structure. However, it also masks unique features of food webs arising when microbial species are included explicitly. There are no "top predators" in food webs containing microorganisms, because all organisms are exploited by parasites of varying lethality. Also, the presence of "three-species loops" has been the subject of controversy in food webs of macroscopic organisms and may be possible only when there is differential predation on species due to developmental stage. In microbial systems this food web structure has not been explicitly investigated, but, since many predators within the system are generalists, it seems likely that such loops can frequently occur due to random encounters.

Food webs including microorganisms must also account for the presence of decomposer organisms. These organisms are not predators because they do not directly impact population dynamics of a prey while obtaining their resources. Decomposer organisms obtain energy or nutrients from previously dead organisms or their by-products. This decomposition is critical to the recycling of nutrients that can be used in primary production. Decomposer organisms affect population dynamics of primary producers by supplying nutrients and often by competing with primary producers for the same resources ("immobilization").

## MUTUALISMS

Mutualisms are interspecific relationships beneficial to both organisms involved. While these relationships have been described as mathematically unstable, a diverse array of cross-kingdom partnerships has existed throughout evolutionary history. Soil mutualists have great impact on above- and belowground community dynamics across a wide range of ecosystems. Organisms in soil collaborate with a wide variety of plants to perform nutrient acquisition services in exchange for plant-derived carbohydrates. While the relationships were originally perceived as bacteria in symbiotic relationships for N acquisition and fungi involved with P acquisition, more recent studies have indicated that fungi are actually involved in the acquisition of almost any limiting nutrient in soil, depending on partnering species (Allen, 1991; Smith and Read, 1997).

Mycorrhiza, the relationship between a plant root and fungus, is one of the most important soil mutualisms. It may be one of the oldest relationships participated in by plants (Brundrett, 2002; Stubblefield and Taylor, 1988). There is evidence that this relationship evolved and was lost multiple times in different divisions in the Kingdom Fungi and in different groups of plants. Mycorrhizal fungi have the ability to acquire nutrients directly from decomposing litter (Leake and Read, 1997) and from live animals such as springtails (Klironomos and Hart, 2001). They can also influence plant–water relations (Allen, 1991) and reduce attack on roots by pathogenic fungi (Gange *et al.*, 1994; Azcon-Aguilar and Barea, 1992). Each of these relationships alters the aboveground community directly by changing rates of reproduction and death of participant species and indirectly by altering competition among plant species.

## ABIOTIC FACTORS

Interactions between organisms have been discussed in terms of resource use. However, environmental modulators (as shown in Table 8.1) can also be affected by organisms. The activities of both nitrifying bacteria and plant roots decrease soil pH, and soil temperature is affected by plant and litter cover. This can have positive or negative impacts on the growth of another species, depending on the species' niche requirements.

Some organisms alter the spatial arrangement of components of the environment or serve as new habitat themselves. These organisms are called "ecosystem engineers" and have widespread effects on an ecosystem beyond their own resource use (Jones *et al.*, 1994). Large, competitively dominant organisms such as trees are obvious examples of ecosystem engineers. Earthworms are ecosystem engineers because they bury plant litter and create macropores in soil.

Many species are commonly found together because they have similar modulator niche requirements or they are adapted to rely on the presence of a common ecosystem engineer. One use of the term "guild" in ecology is to describe such a group of species. Unfortunately, another use of "guild" is to describe species with similar resource requirements, resulting in competition and potentially separated

habitats. Wilson (1999) clarified use of the term by designating the former groups $\beta$-guilds (organisms that commonly occur together because of similar modulator or habitat niche requirements) and the latter group $\alpha$-guilds (organisms that have similar resource requirements and therefore could potentially exclude each other through competition).

## CHANGES IN COMMUNITY STRUCTURE THROUGH TIME AND SPACE

Communities change through a number of processes that can operate over very short to very long time scales. "Succession" is the replacement of populations in a habitat through time due to ecological interactions. A "landscape," in ecology, is the particular spatial arrangement of components of the environment that are important in some way to population dynamics of a given species. Landscapes usually include patches of multiple habitats, as well as variability in conditions that affect habitat quality. Unlike some definitions of the term landscape, this definition does not link landscapes to a particular spatial scale. Instead, it recognizes that landscapes are different for different organisms, depending on the spatial scales over which the organisms interact with the environment (Wiens, 1997). Landscapes have an important impact on local and regional community structure. For example, the structure of a metapopulation (e.g., the number of and distance between populations) is embedded within a landscape.

The habitat that is present in the largest proportion in a landscape and that has the greatest connectivity is considered the habitat "matrix," within which other habitat patches are distributed. A habitat matrix can be occupied by a competitively dominant species or by a diversity of species that coexist through the various mechanisms previously discussed. Alternative nonmatrix habitat patches are created in many ways, and many species are adapted to exploit patchily distributed habitats. The dynamics of these habitats are obviously important to community structure. We have to ask, how are the habitats formed, and what proportion of the landscape do they cover?

One type of nonmatrix habitat is created where the competitively dominant species are absent. This habitat is characterized by an abundance of resources due to lack of competition. Fugitive species are adapted to exploit these patches. A process that causes the removal of an otherwise competitively dominant species or group of species is known as a "disturbance." Disturbances also alter distributions of resources or modulators. Many communities are dependent upon disturbances to maintain species diversity and ecosystem function. Light, for example, determines density and diversity of plants within a stand. In a closed-canopy forest, little light hits the ground. Density is generally high in these stands and diversity low. If these areas are subject to disturbances such as tree fall or fire, density of the stand is decreased, light will strike the forest floor, nutrients and water will not be captured as rapidly, and herbaceous layer species will be allowed to establish.

The initial species to appear after a disturbance are *r*-selected species with dispersal strategies (in space or time) designed to place them in such habitats first. These pioneer species are also capable of making opportunistic use of available resources or have mechanisms to increase rates of nutrient cycling such as N fixation. These species are replaced in time by more competitive species; for example, plant species more tolerant of shade or low soil nutrients. The "climax community" is a stable endpoint of succession, or at least an assemblage in which succession has slowed to the point at which other processes are more important. The initial model of a single climax community has been shown to be inaccurate. Instead, climax communities form a continuum that varies across environmental gradients largely characterized by local variations in climatic and edaphic conditions, local disturbance regimes, and biotic factors, particularly herbivores. Normally the climax community also dominates the landscape and is therefore the matrix community.

Secondary succession follows disturbances that leave soils largely unchanged and plant propagules in the seed bank. The progress of plant succession is often predictable based on climate, soil type, and the presence of seeds in the seed bank. Nutrient cycling is also often altered by disturbances. Enrichment phenomena increase available nutrients, such as by release from litter layers and humic materials through burning. Nutrient availability and rates of nutrient cycles may also be decreased such as when vegetation and humic layers are removed entirely from an area through hurricanes, floods, or intentional management such as plowing. Secondary succession cannot occur following a catastrophic disturbance that removes soil and all biota, such as glacial activity and volcanic eruptions. In this case primary succession, or succession without inputs from a dormant community at the site of the disturbance, occurs at the site. This type of succession often takes hundreds of years to return the community to the predisturbance state. The time required for soil development and recovery of soil populations such as decomposers and mycorrhizal symbionts can often delay recovery of plant communities (Allen *et al.*, 1992).

Disturbances are an inherent part of community structure in a large number of systems. Most disturbances are caused by events that are repeated at some rate and spatial scale. The constant creation of disturbed patches and gradual return to a climax community creates a "shifting mosaic" of different habitats at different stages of succession (Wu and Loucks, 1995). The proportion of the landscape that is in the climax community should equal some steady-state value determined by the rate and spatial scale of the disturbance events and the rate of return to the climax community through succession. This allows fugitive species to depend entirely on the presence of minor habitats with relatively quick turnover rates. Some matrix communities are dependent on widespread repeated disturbance; in this case the matrix community is not the same as the climax community. Such species have evolved mechanisms to persist or regerminate following disturbance. Mangrove forests are dependent on hurricanes to remove colonizing species that without disturbance have the potential to outcompete mangroves. Many grasslands are maintained by fires, as they are responsible for removing tree seedlings that can lead to

the establishment of deciduous forests. Unfortunately, catastrophic events such as fires in areas with high debris loads and in areas unaccustomed to fire, such as the tropical rain forest, result in a great deal of damage because species have not evolved mechanisms to tolerate such disturbances. These events have become more common due to human intervention, as have chronic disturbances such as acid deposition and excessive nutrient loading to which no communities are accustomed.

There are many habitats that are qualitatively different from the matrix habitat and are created through some process other than disturbance. Often an entirely different suite of organisms is adapted to exploit these habitats. Examples include the riparian zone near a river and the river itself. In soil, the rhizosphere, fecal matter, and decomposing plant tissue are important examples of this type of habitat (Blackwood and Paul, 2003). The latter two examples represent habitats defined by a limited pool of resources. Microbial succession in these habitats is driven by a constant change in environmental conditions as resources are used up and the environment is restructured. Some of these habitats, including all the soil habitats in the previous example, are created by events that, like disturbances, have a particular rate of occurrence and spatial scale, followed by community succession. Therefore, they also fit into our model of the landscape as a shifting mosaic of habitats.

The shifting mosaic picture of the landscape is based on a dynamic equilibrium model. However, the particular characteristics of a patch edge, the surrounding habitat patches, and ease of dispersal across other elements of the landscape may all be important determinants of metapopulation and local patch population dynamics. Climate change, human activity, and other novel events can result in nonequilibrium dynamics. Under these conditions, populations are kept from reaching carrying capacity or the stable equilibrium predicted by logistic models.

## HISTORICAL AND GEOGRAPHIC CONTINGENCY

Communities that occupy similar habitats in different regions of the globe normally include different species. Evolution of a new type of organism occurs at a particular location, and the new species must spread out from this location over time. Physical barriers typically prevent the species from colonizing all habitats that could potentially satisfy the species' niche requirements. The diversity of barriers is as rich as that of organisms. The global distribution of a species is both a historical contingency, or dependent on the particular series of events that occurred in the past, and a geographic contingency, or dependent on the particular spatial arrangement of elements of a landscape.

Human activity has greatly increased the transport of materials around the globe. Earthworms from Europe were introduced to the Atlantic coast of North America and have been steadily colonizing new soils each year. The root pathogen *Phytophthora infestans* was introduced from Mexico to the United States and then was transferred to Europe (causing the Irish potato famine) and from there to the rest of the world (Goodwin *et al.*, 1994). Transport of soil is now the subject of international law and regulations. The difference in effects of the introduced species

in these two examples is interesting, given the questions raised above. *P. infestans* in agroecosystems has a substantial impact because it is involved in aggressive exploitation of the dominant plant (an important ecosystem engineer) and clearly causes system reorganization. On the other hand, earthworms play the role of a detritivore involved in comminution of plant tissue. This can be considered a weakly interacting mutualism with plants. Interest in elucidating the ecosystem impacts of introduced soil organisms is increasing as the deleterious effects of the introduction of aboveground species are documented. Interest has also been generated in the relative success rates and characteristics of successful invasive microorganisms as interest in the use of genetically modified organisms has progressed.

## HIERARCHICAL COMMUNITY ASSEMBLY RULES

Community assembly involves a large number of processes and constraints. One area of active research is the development of "community assembly rules," or generalized patterns arising from the operation of ecological mechanisms acting simultaneously in the environment. Belyea and Lancaster (1999) present a model of the hierarchical application of assembly rules (and, in their terminology, constraints) to understand how the assemblage of species present at a given site (the "actual species pool") is selected from all species present in a region (the "total species pool") (Fig. 8.1).

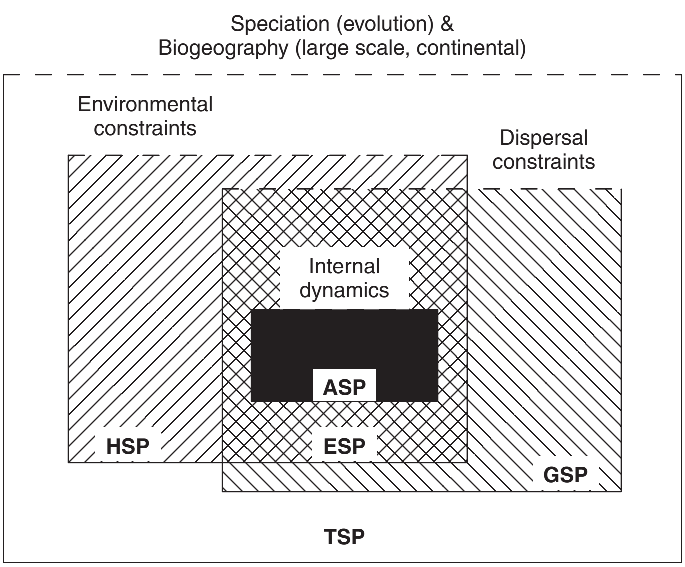



FIGURE 8.1 Hierarchical application of assembly rules and constraints resulting in the assemblage of species present in a habitat (with permission from Belyea and Lancaster, 1999). TSP, total species pool; GSP, geographical species pool; HSP, habitat species pool; ESP, ecological species pool; ASP, actual species pool of a community. See text for description of species pools.

Only a subset of species from the total species pool will be able to disperse to the site in question; this is the "geographical species pool." Composition of the total and geographical species pools is determined by evolutionary history and the particular location relative to distributions of the species; in other words, geographic and historical contingencies. The "habitat species pool" is a subset of the total species pool that could survive at the site based on its environmental characteristics and the species' physiological limits. The overlap between the habitat and the geographical species pools is a set of species called the "ecological species pool." Given enough time, we might expect most species in the ecological species pool to be able to complete their life cycles at a site if they are adapted to the dynamic equilibrium system in place. However, at any given time, the ecological species pool is constrained to the actual species pool by "internal dynamics," which are species interactions such as competition and exploitation.

The hierarchical application of community assembly rules is nicely illustrated by the theory of island biogeography. MacArthur and Wilson's (1967) original theory tried to explain island species richness. Species richness is assumed to be governed by equilibrium between colonization rate of new species and extinction rate of resident species. The habitat species pool can be thought of as the number of appropriate species present on a nearby mainland. The theory is based on the following four assumptions: (1) The immigration rate of nonresident species declines with increasing species richness of the island (the overall immigration rate of species is constant, but the proportion of immigrants that are not already represented on the island changes with species richness of the island). (2) The extinction rate of species on an island increases with increasing species richness (due to increased competition). (3) At a given species richness, the extinction rate of species is lower on larger islands; in other words, a larger island can support greater species richness (due to greater habitat diversity on larger islands and larger populations less prone to stochastic extinction). This is a commonly observed empirical relationship called the "species–area relationship." (4) Immigration rate of new species to an island declines as the island is more distant from the mainland. The first three assumptions are community assembly rules constraining the actual species pool, while the fourth is a rule constraining the geographical species pool. Equilibrium species richness for any island could be predicted by finding the point of intersection between the colonization and the extinction curves given for that island's distance from the mainland and its size (Fig. 8.2).

These rules, and predictions from the theory, have been found valid for many taxa on islands (including some "islands" of terrestrial habitat patches distributed in a substantially different habitat matrix), but not in all cases. Some islands may have not yet achieved equilibrium because of lack of enough time for colonization. Lomolino (1999) presents an alternative theory based on individual species area requirements and dispersal abilities to solve some of these anomalies and predict community composition as well as species richness.

While movement of species to and from islands is an important consideration for determining species composition and density, other considerations such as

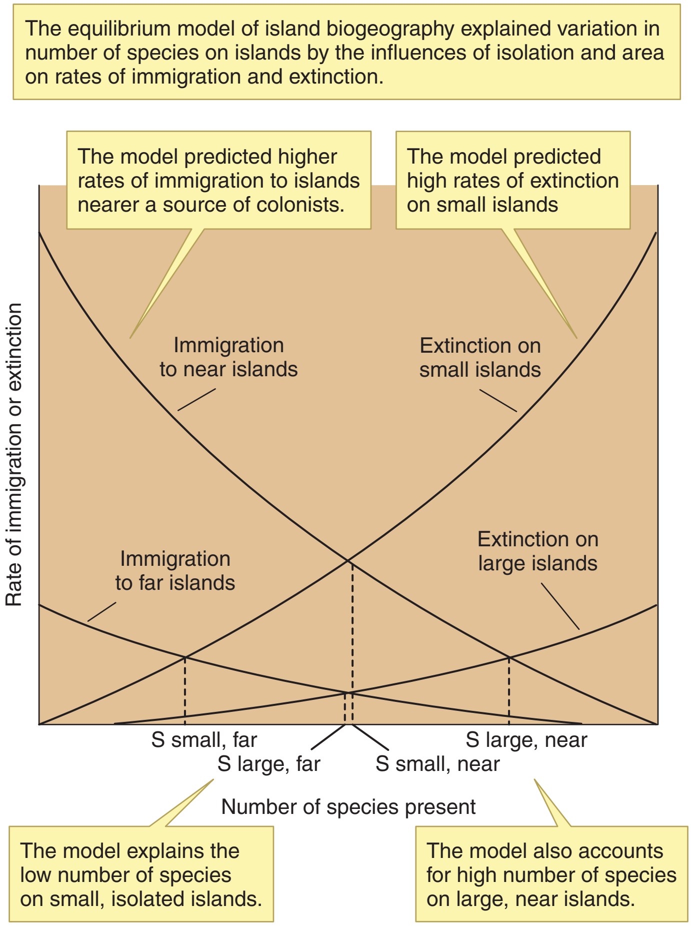



FIGURE 8.2 Island distance and area and rates of immigration and extinction based on the equilibrium model of island biogeography developed by MacArthur and Wilson. Reproduced from Molles (2002) with permission of The McGraw–Hill Companies.

disturbances are also important. Wardle *et al.* (1997) examined 50 islands of varying area in the northern Swedish boreal forest zone. On these islands, area significantly impacted the frequency of fire as larger islands were more often hit by lightning strikes than smaller islands. The result was a difference in plant species composition as a consequence of fire return rate. Smaller islands were more diverse floristically and had more late successional species with poor litter quality compared to larger islands. The differences in fire return rates and aboveground species composition among islands translated into differences in belowground community dynamics on islands of different sizes. Smaller islands had greater humus accumulation, lower microbial activity, and reduced decomposition and N mineralization rates

compared to larger islands. The results suggest that while specific relationships (area, distance from source) govern the number of species in an area, attributes of specific plant species have a great impact on the overall ecosystem function. These results should be considered by conservation ecologists and other resource managers as park systems and reserves operate as islands in a sea of other land use types, and the impact of aboveground management schemes on belowground communities must be considered for maximizing success of the desired outcome.

## ECOSYSTEM DYNAMICS

Ecosystems are systems defined by organisms and the environment within which these organisms interact. They are spatially defined by the interactions of the organisms and their relationship to physical space as an integrated system. The scope of a single ecosystem may extend into the atmosphere or deep into the earth's crust. Ecosystem dynamics are dependent on defined temporal scales. Ecosystems are impacted by long-term and short-term events. Trees are still migrating following the last glacial retreat, altering the components of ecosystems. Humans, which have been on the planet for a very short time, have altered ecosystem dynamics through cultivation and burning and, more recently, globally by increasing soil chemical loads and introducing pesticides and other man-made chemicals. They have also altered the global C cycle by releasing stored C from storage pools. The consequence is an altered planetary climate that will impact most species though altered temperature and moisture regimes.

The specific components of an ecosystem and the controls over those characteristics are largely determined by the "state factors" described originally by Dokuchaev (Jenny, 1961). The state factors include climate, time, parent material, potential biota, and topography. These factors set bounds on the types and rates of processing and the raw materials available for processing within the ecosystem. Climate determines rates of processing by controlling moisture availability and temperature. Time is an important factor for evaluating the degree of weathering of soils or vegetative development since a disturbance. Parent material determines the types of micro- and macroorganisms that can exist and the nutrient and water holding capacity of the medium in which plants must grow. Potential biota includes all organisms that can exist or have existed in an area. For example, deep rooting grasses will differ from other types of plants in their impact on soil development by contributing materials at depth that will be converted into organic matter and turnover slowly. Rooting depth, C:N ratio of materials added to soils, density and diversity of plants, animals, microbes, etc., will all contribute differentially to the soil produced. Finally, topography determines access to water, movement of materials, soil depth, and degree of weathering and can alter plant community structure based on placement along a topographic gradient. Anthropogenic influences have been added to this list of state factors as human impacts on soils can alter the historical development of soils, resulting in soils with novel characteristics.

Discussions of ecosystems within an ecological context focus on energy flows, elemental cycles, and emergent properties. Soil organisms and processes are integral to the development and functioning of all terrestrial ecosystems and, as such, an understanding of soil biology and biochemistry is essential for characterizing ecosystem dynamics. Soil dynamics drive elemental cycles, have controlling roles in ecosystem functions, and are largely determinants of emergent properties (properties not obvious from study of processes at finer levels of organization) such as decomposition rates, nutrient flows, and productivity.

## ENERGY FLOW

The flow of energy in ecosystems is from energy source to autotroph to heterotroph. For most systems, the energy source is the sun and the autotrophs are green plants. The use of energy sources such as inorganic C, N, and S occurs under limited, but not unrealistic, circumstances and is currently of great interest because of the roles of these processes in the production of greenhouse gases such as nitrous oxides and methane. The use of molecules that do not contain C as a source of energy is largely carried out by bacteria and is of great importance for discussing nutrient cycles, but is of lesser importance when discussing energy flow pathways because the contribution to biomass production is a fraction of that produced from solar energy. The solar energy that is captured within an ecosystem is based on the amount of photosynthesis that occurs there. The most commonly used term to begin describing this flow is net primary productivity (NPP), which is the total energy uptake by plants in an ecosystem that is available for use by other trophic levels. NPP is calculated as

$$\text{NPP} = \text{GPP} - R_{\text{p}},$$

where gross primary productivity (GPP) is all of the energy assimilated through photosynthesis, and respiration ($R_{\text{p}}$) is the sum of energy loss through oxidation of organic compounds. The amount of NPP in an ecosystem can be predicted most easily at the largest scale by state factors, but even more simply, patterns of NPP can be described by climate or moisture and temperature alone (Fig. 8.3).

Biomes, the major ecosystem types based on dominant vegetation, are easily plotted along moisture and temperature gradients showing corresponding increases in NPP. At shorter time scales, such as across seasons, NPP is controlled by leaf area, N content, season length, temperature, light, and $CO_2$.

Secondary production, the amount of biomass produced by consumers, is dependent on the amount of energy made available by primary producers. This has consequences for the complexity of trophic dynamics of an ecosystem. The complexity of a food web is likely dictated by the amount of energy that is contained in the producers. The most complex food webs are in the tropical systems where production is not limited by moisture or temperature. Biological systems are inefficient in the transfer of energy. Plants transform only 1 to 5% of the sunlight that is available to them for photosynthesis into biomass. The transformation

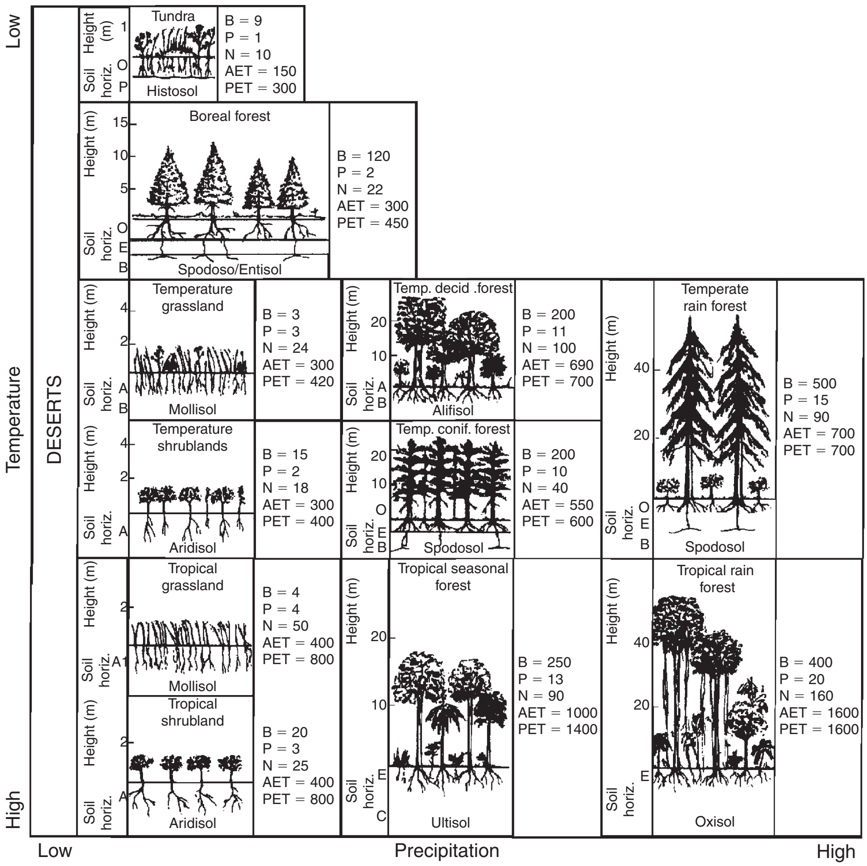



FIGURE 8.3 Modified diagram of the distribution of major ecosystem and soil types in relation to precipitation and temperature. Values for elements of structure and function that are used to characterize ecosystems are given for each type. B, total plant biomass in Mg ha$^{-1}$ y$^{-1}$; P, total plant production above ground in Mg ha$^{-1}$ y$^{-1}$; N, nitrogen uptake by plants in kg ha$^{-1}$ y$^{-1}$; AET, actual evapotranspiration in millimeters of water per year; PET, potential evapotranspiration in millimeters of water per year. Reprinted from Aber and Melillo (2001) with permission from Elsevier.

of plant materials by herbivores into biomass that is available to carnivores is also a relatively inefficient process, with only 10 to 30% of the energy that is available in the plant materials becoming biomass of the herbivores. The energy that is not converted to animal biomass is lost to the environment as a waste product, which is most often heat, satisfying the second law of thermodynamics. The amount of biomass at each level in the food web can be calculated and produces a biomass pyramid characteristic of the ecosystems studied. Biomass pyramids often show the decomposer populations in a separate pyramid or not at all. Little is known about the efficiency with which decomposers recycle detritus. Decomposition of other trophic levels by detritivores significantly impacts the rate at which nutrients

become available for primary producers. While decomposers control the rate at which detrital components such as N and P become available to plants, belowground food webs are responsible for liberating immobilized nutrients. Microbes use energy gained for metabolism, biomass synthesis, and reproduction. The amount of $CO_2$ lost per unit energy gained differs based on the C-use efficiency of the organism. Environmental conditions can impact C-use efficiency (Six *et al.*, 2006). Lack of nutrients, or more importantly, nutrients in specific ratios can alter the amount of energy expended to utilize materials. Some computer models, such as the Century model, assume that of the C assimilated by microbes 55% is lost as $CO_2$, although when nonlignin surface litter is considered, a value of 45% is used (Parton *et al.,* 1987). Substrate quality, nutrient availability, temperature (del Giorgio and Cole, 1998), and protozoan grazing (Frey *et al.*, 2001) impact the C-utilization efficiency of soil organisms. Part of the difficulty in evaluating efficiency is lack of good techniques for assaying efficiency without adding materials to a natural system.

## CARBON, NUTRIENT, AND WATER CYCLES

The term "biogeochemical cycles" emphasizes the intertwined roles of biotic and abiotic components for providing necessary molecules for the growth and reproduction of living organisms. It also emphasizes the importance of soils and soil organisms for driving nutrient availability. The long evolutionary history of microbes has allowed them to develop the machinery necessary to recycle nearly all naturally constructed, complex molecules to the building blocks necessary for the nutrition of higher organisms. The major cycles essential to organisms, and therefore ecosystems, will be described briefly here and are described in more detail elsewhere in this text.

The macronutrients (C, O, H, N, Ca, P, Mg, K, S, Na, Cl) are needed in much greater amounts than the elements considered to be micronutrients (Fe, Mn, B, Co, Cu, Mo, Zn, I, Se). Each element has its own pattern of cycling from the initial stages of solubilization from rock or fixation from the atmosphere ultimately resulting in forms that are available for plant uptake and, finally, recycling by microorganisms. The focus on C, N, and P captures all phases important for biological uptake and represents some of the most biologically important elements.

Flux in the C cycle is largely determined by relative rates of photosynthesis versus respiration and decomposition. Soil organisms and the interactions of soil organisms with each other and plants significantly impact these processes. For example, increases in photosynthesis occur in mycorrhizal plants in nutrient- or water-limited areas. These relationships also alter photosynthetic rates by mediating competition for resources. Soil organisms can impact rates of plant respiration through root predation or other tissue damage. Increased need for tissue repair will result in increased plant respiration as will association with mutualists. Decomposition of plant and animal materials and return of $CO_2$ to the atmosphere are nearly entirely mediated by microbial decomposer communities. The rate at which material is decomposed is impacted, for example, by competition among decomposers

for food resources, predation on decomposers by soil animals or other nonsaprophytes, and alterations to abiotic conditions under which the organisms live.

Human-induced alteration to global fluxes in the C cycle is probably the most important ecological experiment of all time. Responses to human alterations will be determined by the biological responses to elevated $CO_2$, as well as responses to indirect effects such as temperature change and climate instability. The global C cycle (Fig. 8.4) has been greatly altered by the large flux of $CO_2$ into the atmosphere created by fossil fuel use and land use change. The current rate of increase in atmospheric $CO_2$ concentration (+3.2 Pg per year) is a consequence of imbalance between these anthropogenic sources and increased rates of $CO_2$ capture by vegetation, soils, and the ocean (~2 Pg-C per year, not shown). The amount of C moving into soils is mediated by soil organisms. Their contributions to C evolution relative to C storage below ground are of great interest. There are currently a number of studies examining the relative contributions of each belowground participant group to flux (illustrated in Fig. 8.4), C-use efficiency, and the impacts of elevated $CO_2$ and climate change on these fluxes.

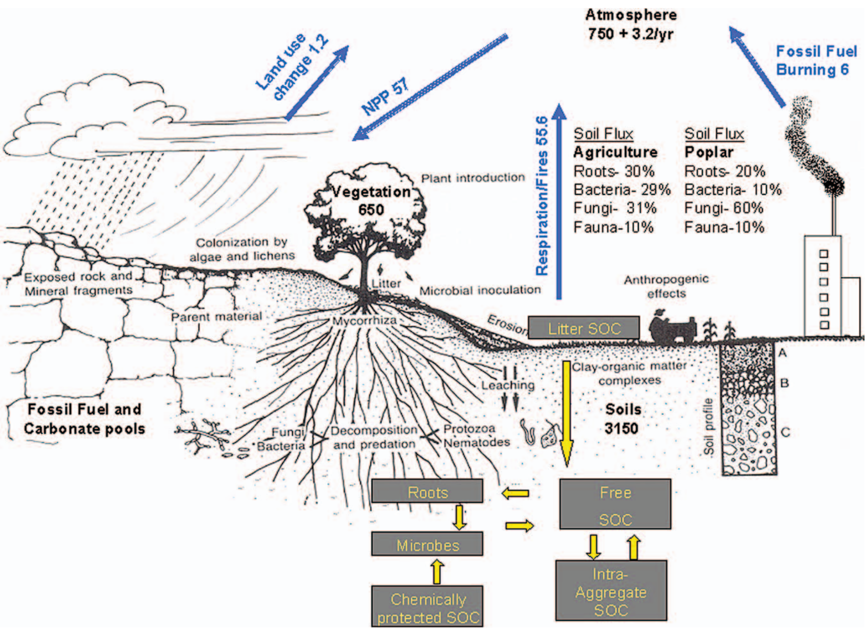



FIGURE 8.4 The global carbon cycle modified from Paul and Clark (1996). Pool and flux values modified from Gruber *et al.* (2004), which included preindustrial values for ecosystem C exchange, are in Pg-C for pools and Pg-C/y for fluxes. Flux values for the global C cycle when summed have failed to account adequately for an increase in atmospheric C of only 3.2 Pg-C/y due in part to high error associated with measured values and poor understanding of all C source/sink mechanisms. The soil fluxes are driven largely by microbial participants, which researchers are currently working to quantify.

Historically, the amount of N that occurred naturally in ecosystems was a consequence of either N fixation by symbiotic or free-living microbes or recycling of organic materials by microbes. This is not now the case. Anthropogenic generation and application of fertilizer N and pollutant dispersal has resulted in a doubling of the amount of N that is currently available for plants in ecosystems. This has changed and will continue to change N availability for plant uptake, the rate at which decomposition occurs, plant species germination and competition, and the degree to which microbes control the amount of N available in terrestrial ecosystems.

The fluxes in the N cycle are primarily driven by N fixation and mineralization (ammonification + nitrification). As such, the microbial community drives key processes in this cycle. Mutualisms with soil organisms such as *Rhizobium* and *Frankia* are important for crops but are also important in providing nutrients to plants in early successional communities where N may not be available. The rate at which N is returned to the system following plant uptake is largely determined by plant form, nutrient-use efficiency, and, ultimately, the rate of mineralization once the organic material is made available to the decomposers. Nitrogen that becomes available for uptake following release from soil organic matter can be taken up either by plants or by soil microorganisms through immobilization, by which available N becomes part of microbial tissues rather than plant tissues.

A great deal of the early literature on the plant–fungal mutualisms focused exclusively on P because mycorrhizas are a key mechanism for movement of P from soil to plants. The need for P in plants is so great and competition so intense that plants with mutualistic relationships can often grow larger than plants without fungi, even though the plants must provide C for fungal growth. As was described for C and N, relationships between soil organisms such as competition, predation, and exploitation determine the rate at which P becomes available for plants and determines relationships within plant communities. The availability of P can also determine the rate of succession so the formation of mutualisms and arrival of decomposer bacteria and fungi has great potential to alter plant community dynamics. Disturbances such as fires that release nutrients to soil can circumvent the need for soil organisms by hastening nutrient availability. According to Liebig's Law of the Minimum, low quantities of any essential nutrient can cause stress and decrease productivity, so the cycling of all nutrients is important in understanding ecosystem dynamics. In most ecosystems, the nutrients that plants depend upon for growth are those that are returned through recycling as a result of microbial action, also called internal cycling or the intrasystem cycle, rather than fresh inputs of nutrients as a result of biotic or abiotic weathering processes.

The water cycle is the only cycle that does not fit the generalized schemes described above for element cycling. It is more of a physical process than a chemical cycle. Solar energy is largely responsible for the evaporation of water from the ocean. Only about 10% of this water then precipitates onto land, with the remainder returning to the ocean. Water is transported in clouds over the land where it is joined by water released from terrestrial surfaces through evapotranspiration by plants. This water can then be returned to the earth's surface through precipitation events.

The timing of precipitation events is determined by topography, temperature, and relative humidity. Water that strikes the earth's surface can infiltrate and percolate in soil, which can allow groundwater recharge. Alternatively, water can runoff and contribute to water bodies such as rivers and streams. Runoff can have negative impacts on soil quality as increased runoff rates due to poor management or alterations to vegetation can result in loss of soil and soil nutrients. The water cycle is particularly important in terms of ecosystem dynamics because this cycle purifies water. Water that evaporates from plant surfaces and streams does not contain contaminants so the rainwater that returns to the surface of the planet is of much higher quality than the water found on the surface. Unfortunately anthropogenic sources of pollution now contaminate the precipitation as it returns to the surface.

## EMERGENT PROPERTIES

Emergent properties of ecosystems are a consequence of the synergistic effects of community composition on "ecosystem function" (flux of energy and materials through the ecosystem). The idea that the forest is more than the trees conveys the importance of the concept, but it can be applied broadly to every ecosystem. Species' impacts on turnover rates and productivity have profound effects. As a very simple example, Blair *et al.* (1990) found that while decomposition rates for mixed litter were similar to the decomposition rates for the litter of tree species incubated individually, there were significant alterations to N flux and abundance of decomposer organisms that could not be predicted based on the patterns detected in incubations of individual litter types. Indeed, one of the most hotly debated and difficult relationships to define is that of the importance of biodiversity to ecosystem function.

There have been a number of hypotheses developed to explain the inherent importance of biodiversity, but few studies have provided convincing support for these hypotheses. Questions such as whether biodiversity impacts characteristics including ecosystem productivity, nutrient retention, and stability have permeated the literature over the past several decades. In an era of decreasing global biodiversity and decreasing genetic diversity, these questions require immediate attention. Intellectually there is no denying that maintaining biodiversity is essential to maintaining the integrity of systems. There are two main problems with this challenge. The first is that measuring the complete biodiversity of a system and determining the degree to which it is in an "undisturbed state" is difficult. Second, biodiversity is not a term that is indicative of quality but quantity. Increasing diversity by increasing undesirable species such as nonnative invasive species will not maintain the integrity of a system, so biodiversity for biodiversity's sake is not the answer. So, what species or how many species should an ecosystem contain? The example above of Blair *et al.* (1990) suggests that species interactions impact ecosystem function, in this case N flux within a system, which will feed back to impact energy allocation to nutrient acquisition by the plant. Tilman (1996) demonstrated that ecosystem stability increased with aboveground species diversity. However, Klironomos *et al.* (2000) found that the presence or absence of arbuscular mycorrhizal (AM) fungi

significantly changed the relationship of plant diversity to aboveground productivity. Without the fungi, productivity increased as plant species were added to a total of 15 species in a linear fashion. Productivity was maximized at 10 plant species in an asymptotic fashion with AM fungi present. The results suggest that considerations of the importance of diversity in ecosystem function must include an understanding of diversity of all participants, otherwise the importance of diversity among different components may be overlooked. In contrast, the island biogeography study by Wardle *et al.* (1997) described above found the islands with the greatest aboveground diversity had lower ecosystem process rates. Whether lower rates of nutrient cycling and decomposition are tied to lower belowground diversity remains to be seen.

One of the biodiversity hypotheses examines the degree to which there is functional redundancy in microbial communities. This hypothesis suggests that there are so many species that have the same function that loss of one will not alter the way that the system operates. There are no studies that can provide adequate support for or can eliminate this hypothesis. The amount of stress placed on ecosystems across the globe by anthropogenic influences has changed the way ecosystems operate. Whereas there may have been no impact of the loss of one species from a system before anthropogenic N deposition, this chronic disturbance may have changed the response of the system to the loss of one species. There are two main terms used to characterize the way that an ecosystem responds to a disturbance. While a system that does not change appreciably following a disturbance is said to be resistant, a system that changes but returns to its predisturbance state within a reasonable time frame is said to be resilient. The degree to which ecosystems are resistant or resilient may depend entirely on biodiversity and on the amount of stress currently on the system. Unfortunately, as much as this information is needed to protect ecosystems from degradation and protect species against loss, the stability of ecosystems is an emergent property that cannot easily be measured quantitatively as a parameter or by quantifying one or more of its component parts.

## CONCLUSION

Integrating across scales is a challenge that ecologists have faced for the past hundred years. Scientists that examine microorganisms or soil communities have always dealt with this particular issue. For example, how does one acquire soil samples, in a forest, that are representative of the organisms that live there, in a manner that will allow one to evaluate treatment differences or small-scale rates of change? Conversely, present interpretation of global-scale processes such as net primary productivity and C cycling require very little understanding of microbial community dynamics. Processes such as nutrient cycling impact site productivity, but productivity at a global scale can be predicted based on temperature and moisture patterns alone. In models, the microbially mediated steps of nutrient cycling are also predicted by patterns of temperature and moisture. The dilemma

facing scientists is that if one considers the component parts of any elemental cycle, the rate that a nutrient becomes available for uptake is dependent on the life cycle of a soil bacteria or the reach of a mycorrhizal companion. In other cases, such as where there is a unique biological interaction (e.g., pathogenesis, mutualism, ecosystem engineering), prediction of population and community dynamics is critical. Failure to investigate the causes and impacts of microbial community structure over the long term will retard our ability to manage ecosystems for the greatest benefit to society and reduce our understanding of the impacts of species loss and global climate change. The role that each organism plays must be examined across spatial scales from molecules to ecosystems, and temporal scales from seconds to centuries, or our ability to predict problems or mitigate damage will be impaired. Integration across scales is a challenge that those that study belowground systems can begin to facilitate.

The field of science known as ecology is more integrative than most other fields. Ecologists are dependent on specialists that reach across the breadth of the physical sciences. Scientists that study soil microbiology and biochemistry can contribute to and benefit from approaching the medium and organisms they examine as ecologists. Society has placed a great burden on scientists by damaging systems before understanding how they operate. Science is now charged with developing an understanding of these systems and finding ways to mitigate the damage. This can be accomplished only by integrating across scientific fields. As such, this brief introduction to ecology was prepared to stimulate an awareness of the contributions that studies of soils have already made to our understanding of the operation of the natural world and the need to continue to integrate scientific endeavors from molecules to the biosphere.

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# 9

# The Physiology and Biochemistry of Soil Organisms

W. B. McGill



## INTRODUCTION

Green plants release $O_2$ during photosynthesis. Decomposition consumes $O_2$ and the cycle is complete. This is good. Why then has $O_2$ accumulated in the earth's atmosphere? If $O_2$ can accumulate, why is there not more of it? With an atmosphere having nearly 21% by volume of $O_2$ in the atmosphere, why does all S not accumulate as $SO_4^{2-}$, or N as $NO_3^-$, which are the thermodynamically stable species of these elements? How is it that $S^{2-}$ can be oxidized to $S^0$ or $SO_4^{2-}$ under anaerobic (i.e., reducing) conditions? How can highly aromatic compounds such as aniline be metabolized under anaerobic conditions, and why would such a capability develop in soils? Is it that many of the earth's functions do not make sense from a thermodynamic or mass balance perspective? Or do they? The answers arise from three things: first, the unique attributes of soils as habitats in which aerobic and anaerobic microsites coexist due to spatial heterogeneity over distances of millimeters or less (see Chaps. 2 and 8) and where aerobic and anaerobic conditions alternate over time. So both spatial and temporal changes in soil habitats are involved. Second, the unique range of physiological capabilities of soil organisms

allows them to gain energy by a wider range of mechanisms than exist in higher organisms. Third, mechanisms operating at long (geologic in some cases) time scales sequester substrates in locations isolated from active microorganisms.

Soils are nature's integrators; they form on the surface of the earth where the cycles of matter and the transfer of energy all meet. Soils interact reciprocally with the biosphere, hydrosphere, lithosphere and atmosphere (Fig. 9.1).

Only in soils do all four fundamental spheres interact. Such interactions entail biological, chemical, biochemical, and physical transformations and biological and physical translocations. Soil organisms and especially soil microorganisms are intimately involved in biological and biochemical transformations. They are both sinks for elements and catalysts to speed transformations of elements. Hence physiology and biochemistry of soil organisms is fundamentally important to understanding earth systems. Such a myriad of interactions may appear chaotic, random, or complex beyond understanding. Not so. Nature may be complex but systematic, and ways exist to organize knowledge. The requirement to conserve mass means that earth systems must operate in cyclic ways, so cycles become helpful organizing principles. Additionally size scales and time scales are related. A way to look at this relationship is through the lens of cycles embedded within cycles. The shorter the cycle, and the smaller the mass of material within it, the faster it must operate to connect to a larger cycle with a greater mass of material moving through it, but at a slower pace. Three groups of cycles are useful: geochemical cycles, biogeochemical cycles, and biochemical cycles. As used here, geochemical cycles are analogous to macroscale systems, are dominated by exchange of materials among ecosystems, and are represented by processes occurring in zonal soils, and on a global scale they represent cycles of volcanism, weathering, and erosion. Biogeochemical

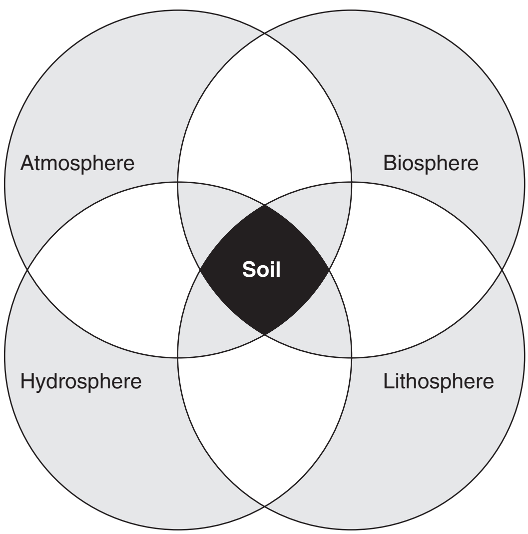



FIGURE 9.1 Soil, or the pedosphere, as the integration of four fundamental spheres. (Reproduced from McGill (1996), with permission from SBCS and SLCS.)

cycles relate to mesoscales, typically exchange materials within ecosystems, are dominant in soil landscapes, and are represented by element cycles such as the N cycle. Biochemical cycles operate within individuals or single cells, come close to the microscale, and dominate within soil profiles or aggregates and can be represented by the tricarboxylic acid cycle (TCA cycle).

## METABOLIC CLASSIFICATIONS OF SOIL ORGANISMS

How can organisms, their physiology, and their functions be organized? Criteria with which to classify soil microorganisms include size, morphology, molecular genetics or physiology, and metabolism. A metabolic classification based on source of energy, electrons, and C has practical value (Tables 9.1 and 9.2). Phototrophs

TABLE 9.1 Metabolic Classification of Soil Microorganisms

| Metabolic group | Electron source | Electron acceptor[a] | C source |
|---|---|---|---|
| | Energy from light (ATP generated by photosynthesis) | | |
| Photolithotroph | Reduced minerals | $NAD^+$ | $CO_2$; some organics |
| Photoorganotroph | Organic C compounds | $NAD^+$ | $CO_2$; some organics |
| Photoaquatroph | $H_2O$ | $NAD^+$ | $CO_2$ |
| | Energy from chemical oxidations | | |
| Chemolithotroph | Reduced minerals or inorganic ions | $O_2$; more oxidized minerals or ions | $CO_2$ |
| Chemoorganotroph | Organic C compounds | $O_2$; organic compounds; minerals or inorganic ions | Organics |

[a]Terminal electron acceptor at end of respiratory chain or apparatus to synthesize ATP.

TABLE 9.2 Representative Examples of Reactions Mediated by Some Metabolic Groups of Soil Microorganisms

| | Energy source | |
|---|---|---|
| Electron source | Light | Chemical |
| Inorganic | Photolithotrophs, e.g., purple S bacteria $2H_2S + CO_2 \rightarrow 2S^0 + CH_2O + HOH$ | Chemolithotrophs, e.g., nitrifiers[a] $30NH_4^+ + 34O_2 + 26CO_2 \rightarrow 30NO_3^- + 26CH_2O + 60H^+ + 4\ HOH$ |
| Organic | Photoorganotrophs, e.g., purple non-S bacteria $3CH_2O + 2N_2 \rightarrow 3CO_2 + 4NH_3$ | Chemoorganotrophs, e.g., decomposers $CH_2O + O_2 \rightarrow CO_2 + HOH$ |
| Water | Photoaquatrophs, e.g., higher plants $HOH + CO_2 \rightarrow O_2 + CH_2O$ | |

[a]Stoichiometry from Eqs. [18] and [19] of Table 9.10 and Table 9.11. (Reproduced from McGill (1996), with permission from SBCS and SLCS.)

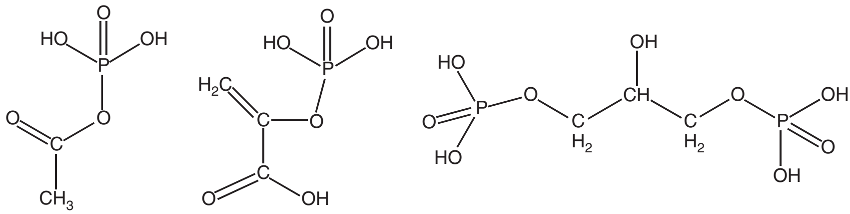



FIGURE 9.2 Phosphorylated compounds involved in substrate-level phosphorylation.

use light as an energy source; in contrast, chemotrophs use chemical forms of energy. These fundamental groups may be subdivided into organotrophs, for which the electron source is organic; lithotrophs, for which the electron source is inorganic; or aquatrophs, for which it is water. Organisms that use an organic form of C are referred to as heterotrophs; those that use $CO_2$ as C source are called autotrophs. Most heterotrophs are chemoorganotrophs (e.g., *Pseudomonas* spp., but some such as Rhodospirillaceae are chemophototrophs). Most autotrophs are chemolithotrophs or photolithotrophs. Some, such as the $H_2$-oxidizing bacteria ($6H_2 + CO_2 + 2O_2 \rightarrow CH_2O + 5H_2O$), are facultative chemolithotrophs capable of growing on carbohydrates under aerobic conditions (see Gottschalk, 1986).

## ELECTRONS AND ATP

All organisms use the energy associated with the reaction ATP $\rightarrow$ ADP + $P_i$ to activate reactions needed for growth and reproduction. What is the source of the ATP? Formation of ATP occurs by two routes: (a) substrate-level phosphorylation or (b) electron transport phosphorylation.

## SUBSTRATE-LEVEL PHOSPHORYLATION

Substrate-level phosphorylation is most significant in anaerobes, and yields of ATP are low. Consequently production of biomass is low and the production of fermentation intermediates for downstream metabolism by other microbes is high. Oxidation of organic molecules (removal of $e^-$ and $H^+$) allows incorporation of inorganic phosphate and formation of a phosphorylated intermediate. Examples of such intermediates include 1,3-bisphosphoglycerate and phosphoenolpyruvate, both in the Embden–Myerhof–Parnas pathway, plus acetyl phosphate, in anaerobes that form acetate (Fig. 9.2). Hydrolysis of the phosphoryl group of these intermediates releases enough energy to form ATP when they are coupled with ADP and $P_i$. For example, see Fig. 9.3, in which acetate kinase catalyzes the transfer of a phosphoryl group from acetyl phosphate to ADP $\rightarrow$ ATP.

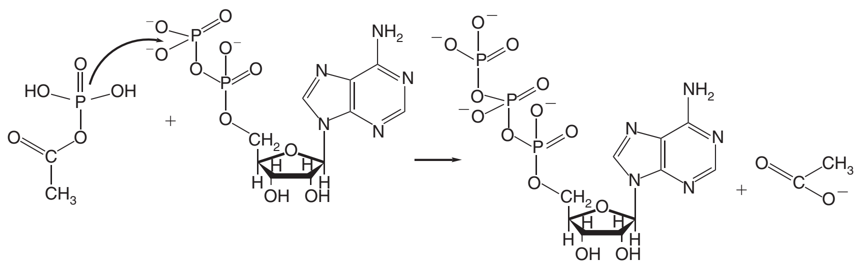



FIGURE 9.3 Example of substrate-level phosphorylation using acetyl phosphate.

## ELECTRON TRANSPORT PHOSPHORYLATION

Electron transport phosphorylation is more important than substrate level phosphorylation in phototrophs and aerobic and facultatively anaerobic chemotrophs. It occurs during respiration and photosynthesis. During C metabolism by chemoorganotrophs, NADH and FADH are produced from oxidation of organic substrates, for example in the TCA cycle. Electron transport phosphorylation entails transfer of electrons from donors such as NADH (or FADH) with a negative redox potential to acceptors such as $O_2$ with a less negative or positive redox potential. The associated energy change is coupled to the phosphorylation of ADP + $P_i \rightarrow$ ATP. Electron transport phosphorylation is associated with membranes.

Given that ATP is used to energize soil microbial biomass formation, how much ATP is produced during oxidation of a fixed amount of substrate? Understanding the relationship between substrate oxidized, $O_2$ consumed or electrons ($e^-$) transferred, and ATP formed helps us understand carbon utilization efficiency (CUE; ratio of C consumed/C converted to biomass) on one hand or quantities of alternate electron acceptors needed on the other. The CUE in turn helps regulate elemental dynamics; for example, N mineralization increases and N immobilization decreases as CUE decreases. Similarly, as the amount of ATP produced per mole of $e^-$ decreases, there is an associated increase in the number of moles of $O_2$ needed to generate a fixed amount of ATP, or alternate electron acceptor (e.g., $NO_3^-$), needed in the absence of $O_2$.

Under aerobic conditions, each mole of NADH or FADH carries $2e^-$ and each $2e^-$ reduce one atom of O. Therefore under aerobic conditions ATP production from electron transport phosphorylation with $O_2$ as the electron acceptor can be equated to electrons released or atoms of O consumed. If O is not the terminal electron acceptor, then ATP production from electron transport phosphorylation can be equated only to electrons released. The ratio of ATP production to $e^-$ released or atoms of O consumed may be designated the P/O or P/$2e^-$ ratio, under aerobic conditions, or P/$2e^-$ ratio when $O_2$ is not the terminal electron acceptor.

The P/O or P/2$e^-$ ratio may be estimated using either a general rule or slightly more detailed calculations. We will consider the general rule first, followed by a more detailed example. Generally the oxidation of 1 mol of NADH is considered to generate 3 mol of ATP, and oxidation of 1 mol of FADH generates 2 mol of ATP. Expressed as a ratio of ATP/NADH or ATP/FADH one gets:

$$\frac{3ATP}{NADH} \text{ and } \frac{2ATP}{FADH}.$$

Expressed as P/O ratio the above ratios become:

$$\frac{P}{O} = 3 \text{ for NADH + H}^+ \text{ oxidized by O}_2 \text{ and}$$

$$\frac{P}{O} = 2 \text{ for FADH + H}^+ \text{ oxidized by O}_2.$$

Consider the TCA cycle, which generates 3 NADH + $H^+$, 1 FADH + $H^+$, and 1 ATP. According to the general rule the expected ATP production in the TCA cycle becomes:

| | |
|---|---|
| From NADH + $H^+$: | $3 \times 3 = 9$ mol ATP |
| From FADH + $H^+$: | $1 \times 2 = 2$ mol ATP |
| Substrate-level phosphorylation: | $1 = 1$ mol ATP |
| Total: | 12 mol ATP per turn of the cycle |

Each turn of the cycle releases 2 mol of $CO_2$, consumes 2 mol of $O_2$ or 4 atoms of O, and releases 8 electrons. Given the classical concept that each turn of the cycle produces 12 mol of ATP as above, the classical P/O ratio for the entire TCA cycle becomes:

$$\frac{P}{O} = \frac{12ATP}{4O} = 3.$$

The above general simplification, however, does not accommodate the variety of arrangements that may exist in membranes for electron transport phosphorylation. Therefore a more detailed or mechanistic calculation can be based on the chemiosmotic theory (Fig. 9.4).

According to this theory electrons are passed from donors such as NADH to redox receptors in respiratory complexes within the inner membrane of mitochondria or the plasma membrane of bacterial cells. The redox receptors are connected in a series of couples within up to four of these complexes. Reduction of some redox receptors draws an $H^+$ ion from the cytoplasm (n phase of the membrane). As it flips to the reduced state, the $H^+$ is extruded to the periplasmic space (p phase), thereby increasing the $H^+$ concentration in the periplasm. As this process continues a gradient of $H^+$ develops across the membrane with a high

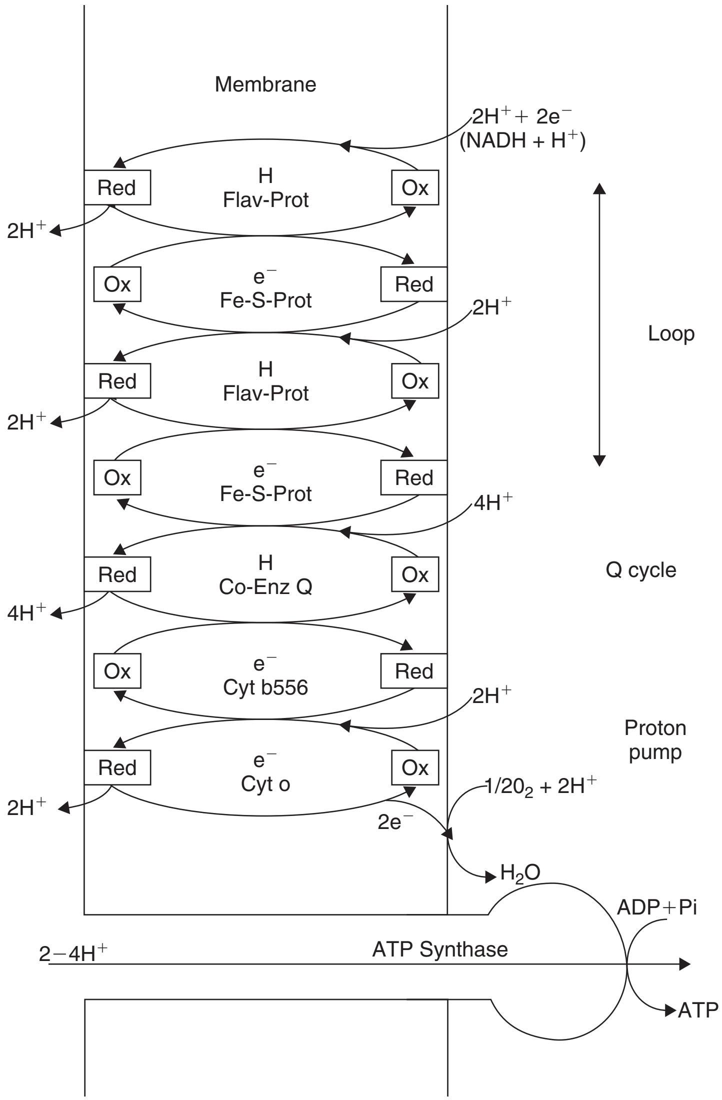



FIGURE 9.4 Schematic outline of the functional arrangement of components of the electron transport chain in the inner membrane according to the chemiosmotic theory. The "loop," "Q-cycle," and "proton pump" mechanisms for $H^+$ exclusion are represented. Not all parts are always present. This would be typical of many aerobic chemoorganotrophic soil microorganisms. For further details see Gottschalk (1986) and Nicholls and Ferguson (1992).

concentration in the p phase compared to the n phase. Consequently an electrical potential, or proton motive force ($\Delta P$), is developed to drive $H^+$ from the p to the n phase through the ATP synthase complex. (Think of all those lonely expelled $H^+$ ions anxious to get back to their mates.) The energy released in this process is

used to form ATP from ADP. Consequently the production of ATP per mole of $O_2$ consumed or per $2e^-$ released is determined first by the number of redox couples in the membrane, which determines the moles of $H^+$ extruded to the p phase, and second by the number of moles of $H^+$ that must be driven from the p to the n phase through the ATP synthase complex to produce a mole of ATP. The product of these two ratios determines the actual P/O ratio:

$$\frac{P}{O} = \frac{H^+}{2e} \times \frac{ATP}{H^+}. \tag{1}$$

The electron transport chain contains up to four complexes involved in $H^+$ extrusion and electron transport. The number of such complexes through which the electrons flow for a particular electron source determines the value of the $H^+/2e^-$ ratio as schematically represented in Fig. 9.4. The $H^+/2e^-$ ratio appears to vary from 10 for NADH oxidized by $O_2$ to 6 for FADH oxidized by $O_2$ (Nicholls and Ferguson, 1992). Considering that the ATP/$H^+$ ratio appears often to be close to 4 (Nicholls and Ferguson, 1992) we can calculate the P/O ratio using Eq. (1). Consequently the P/O ratio may be:

$$\frac{P}{O} = \frac{H^+}{2e} \times \frac{ATP}{H^+} = 10 \times \frac{1}{4} = 2.5 \quad \text{for NADH + H}^+ \text{ oxidized by O}_2 \text{ and}$$

$$\frac{P}{O} = \frac{H^+}{2e} \times \frac{ATP}{H^+} = 6 \times \frac{1}{4} = 1.5 \quad \text{for FADH + H}^+ \text{ oxidized by O}_2.$$

The ATP produced per turn of the TCA cycle can now be calculated using these values derived from the more mechanistic chemiosmotic theory. Specifically we now get $3 \times 2.5 = 7.5$ mol of ATP from NADH + $H^+$, $1 \times 1.5 = 1.5$ mol ATP from FADH + $H^+$, and 1 mol of ATP from substrate-level phosphorylation. Given the values above from the chemiosmotic theory each turn of the cycle produces $7.5 + 1.5 + 1 = 10$ mol of ATP per turn of the TCA cycle. The more detailed P/O ratio for the entire TCA cycle now becomes:

$$\frac{P}{O} = \frac{H^+}{2e} \times \frac{ATP}{H^+} = 6 \times \frac{1}{4} = 1.5\,.$$

## OVERVIEW OF MECHANISMS TO GENERATE ATP AND REDUCING EQUIVALENTS

Four variations on the theme in Fig. 9.4 are used to generate ATP and reducing equivalents in phototrophic and aerobic chemotrophic organisms (Fig. 9.5).

In Fig. 9.5A, electron transport through the respiratory chain to $B_{ox}$ generates the $\Delta P$ (proton motive force) needed to synthesize ATP (e.g., chemoorganotrophs such

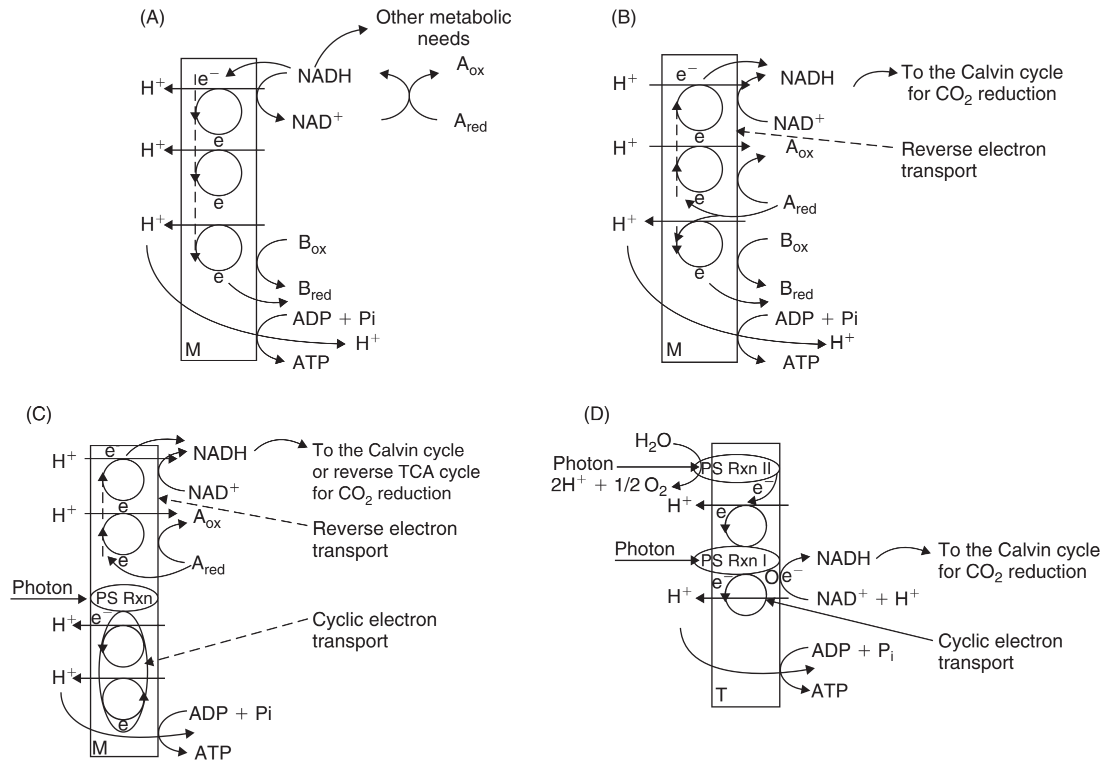



**FIGURE 9.5** Generation of ATP and reducing equivalents (NADH) in: (A) chemoorganotrophs in which substrates are oxidized within the cytoplasm (e.g., *Pseudomonas* spp.); (B) chemolithotrophs in which substrates are oxidized at the membrane (e.g., *Nitrobacter* spp.); (C) anoxygenic phototrophic bacteria, Rhodospirillaceae (purple nonsulfur bacteria), Chromatiaceae (purple sulfur bacteria), Chlorobiaceae (green sulfur bacteria), and Chloroflexaceae (green gliding bacteria), when oxidizing substrates such as $H_2$; or (D) oxygenic phototrophic cyanobacteria. Small circles within the plasma membrane (M) or thylakoid membrane (T; an intracytoplasmic membrane) represent redox complexes. PS Rxn, photosynthesis reaction site.

as *Pseudomonas* spp.). Reducing equivalents (NADH) are produced by oxidation of substrates within the cytoplasm. In Fig. 9.5B, reverse electron transport is used to generate NADH, which is used to reduce $CO_2$ in the Calvin cycle. Electron transport through the respiratory chain to $B_{ox}$ generates the $\Delta P$ needed both to synthesize ATP and to drive reverse electron transport (e.g., chemolithotrophs such as *Nitrobacter*).

Photosynthesis is the ultimate source of energy to allow soil organisms to work. Photosynthesis requires no oxygen (although it may partially cycle $O_2$) and may or may not generate $O_2$. If photosynthesis uses HOH as an electron donor, as it does for photoaquatrophs, then it generates $O_2$ and is called oxygenic photosynthesis (Table 9.3; Eq. [1]).[1] Photosynthesis that uses a reduced mineral such as $H_2S$ as electron donor, as is the case with photolithotrophs, produces $S^0$ (Eq. [2]) (or $SO_4^{2-}$; Eq. [3]), which is called anoxygenic photosynthesis (Staley and Orians, 1992).

All anoxygenic photosynthetic organisms represented in Fig. 9.5C can use $H_2$ as H donor; in addition the purple nonsulfur bacteria (Rhodospirillaceae) and the green gliding bacteria (Chloroflexaceae) can use organic substrates, the purple sulfur bacteria (Chromatiaceae) can use $H_2S$ and organic substrates, and the green sulfur bacteria (Chlorobiaceae) can use $H_2S$, but not organic substrates. Photon activation of electrons in the photosynthesis reaction site (PS Rxn) generates cyclic transfer of electrons through a series of redox couples, resulting in formation of ATP, and eventually the electron returns to its ground state and to the PS Rxn site. $NAD^+$ is reduced using reverse electron transfer driven by $\Delta P$ generated from photosynthesis. Subsequent transhydrogenation allows NADH to reduce

TABLE 9.3 Anoxygenic and Oxygenic Photosynthesis

| | | | | |
|---|---|---|---|---|
| | Oxygenic photosynthesis, e.g., *Panicum, Nostoc* | | | |
| | $h\upsilon + 2HOH + 3(ADP + P_i)$ | $\rightarrow$ | $4e^- + 4H^+ + O_2 + 3ATP$ | |
| | $3ATP + CO_2 + 4e^- + 4H^+$ | $\rightarrow$ | $CH_2O + HOH + 3(ADP + P_i)$ | |
| Overall: | $h\upsilon + CO_2 + HOH$ | $\rightarrow$ | $CH_2O + O_2$ | [1] |
| | Anoxygenic photosynthesis, e.g., *Chromatium* (purple S bacteria) | | | |
| | $2H_2S$ | $\rightarrow$ | $2S^0 + 4e^- + 4H^+$ | |
| | $h\upsilon$ + pigment | $\rightarrow$ | $[\text{pigment}^+ + e^-]^a$ | |
| | $3(ADP + P_i) + [\text{pigment}^+ + e^-]^a$ | $\rightarrow$ | 3ATP + pigment | |
| | $3ATP + CO_2 + 4e^- + 4H^+$ | $\rightarrow$ | $CH_2O + HOH + 3(ADP + P_i)$ | |
| Overall: | $h\upsilon + CO_2 + 2H_2S$ | $\rightarrow$ | $CH_2O + 2S^0 + HOH$ | [2] |
| Or overall: | $h\upsilon + 3CO_2 + 2S^0 + 5HOH$ | $\rightarrow$ | $2SO_4^{2-} + 3CH_2O + 4H^+$ | [3] |

[a]Complex including several components of the photosynthetic system and the respiratory chain. (Reproduced from McGill (1996), with permission from SBCS and SLCS.)

[1]Numbers in square brackets refer to equation numbers in the tables.

$NADP^+$ to NADPH, which is used to reduce C in the Calvin cycle (Nicholls and Ferguson, 1992). In the Chlorobiaceae $CO_2$ is reduced by reversal of the TCA cycle, all other anoxygenic photosynthetic organisms use the Calvin cycle (see Gottschalk, 1986, for additional reading). NADH is used to reduce $CO_2$, so the electron and H transfer to $CO_2$ in anoxygenic phototrophic bacteria can be summarized by Eq. [2] in Table 9.3.

In oxygenic photosynthesis (Table 9.3, Eq. [1]) ATP is synthesized during $e^-$ transfer from HOH to $NADP^+$, and pseudocyclic $e^-$ transfer without formation of NADPH meets additional ATP needs (Nicholls and Ferguson, 1992). The reducing equivalents (NADPH + $H^+$) are used in the Calvin cycle for reduction of C. In Fig. 9.5D the electrons released from splitting of water are passed linearly to $NAD^+$ along a series of redox couples during which $\Delta P$ is generated for ATP synthesis. Two PS Rxn sites are involved. This linear transfer of electrons may not generate enough $\Delta P$ to produce enough ATP for $CO_2$ reduction. Cyclic electron transfer can make up the shortfall.

## EXAMPLES OF SOIL MICROBIAL TRANSFORMATIONS

### NITROGEN FIXATION

After C fixation through photosynthesis, $N_2$ fixation is the second fundamental reaction needed to introduce essential elements from the atmosphere to the pedosphere (Table 9.4). Representatives of both the photoaquatrophs (Eq. [4] in Table 9.4)

TABLE 9.4 Dinitrogen Fixation Accompanying Anoxygenic and Oxygenic Photosynthesis

| | | | |
|---|---|---|---|
| Oxygenic photosynthesis and $N_2$ fixation, e.g., *Nostoc* | | | |
| $h\upsilon + 6HOH + 19(ADP + P_i)$ | → | $3O_2 + 12e^- + 12H + 19ATP$ | |
| $3ATP + CO_2 + 4e^- + 4H^+$ | → | $CH_2O + HOH + 3(ADP + P_i)$ | |
| $12ATP + N_2 + 6e^- + 6H^+$ | → | $2NH_3 + 12(ADP + P_i)$ | |
| $4ATP + 2e^- + 2H^+$ | → | $H_2 + 4(ADP + P_i)$ | |
| Overall: $h\upsilon + 5HOH + CO_2 + N_2$ | → | $3O_2 + CH_2O + 2NH_3 + H_2$ | [4] |
| Anoxygenic photosynthesis and $N_2$ fixation, e.g., *Chromatium* (purple S bacteria) | | | |
| $6H_2S$ | → | $6S^0 + 12e^- + 12H^+$ | |
| $h\upsilon$ + pigment[a] | → | [pigment$^+$ + $e^-$][a] | |
| $19(ADP + P_i)$ + [pigment$^+$ + $e^-$][a] | → | 10ATP + pigment[a] | |
| $3ATP + CO_2 + 4e^- + 4H^+$ | → | $CH_2O + HOH + 3(ADP + P_i)$ | |
| $4ATP + 2e^- + 2H^+$ | → | $H_2 + 4(ADP + P_i)$ | |
| $12ATP + N_2 + 6e^- + 6H^+$ | → | $2NH_3 + 12(ADP + P_i)$ | |
| Overall: $h\nu + 6H_2S + CO_2 + N_2$ | → | $6S^0 + CH_2O + 2NH_3 + H_2 + HOH$ | [5] |

[a]Complex including several components of the photosynthetic system and the respiratory chain. (Reproduced from McGill (1996), with permission from SBCS and SLCS.)

and the photolithotrophs (Eq. [5]) carry out $N_2$ fixation, but it is not restricted to phototrophs. Consequently, both the oxygenic and the anoxygenic forms of photosynthesis are associated with the ability to fix $N_2$ as well as to create reduced C for release to subsequent organisms along the food chain. The amount of $N_2$ fixed per mole of $e^-$ generated varies because nitrogenase also coreduces $H^+$ to $H_2$ in varying amounts. Similarly the ATP/2$e^-$ ratio varies (Paul and Clark, 1989). The values for number of ATPs produced in Table 9.4 are based on 1 mol of $H_2$ generated per mole of $N_2$ fixed and an ATP/2$e^-$ ratio of 4.

## AEROBIC CHEMOLITHOTROPHIC EXAMPLES

The aerobic chemolithotrophic group contains organisms with the ability to reduce $CO_2$ to $CH_2O$ using the Calvin cycle and energy derived from oxidation of reduced minerals. It cannot operate independent of oxygenic photosynthesis, however, because of its strict requirement for $O_2$.

### Oxidation of $H_2S$ with Reduction of $CO_2$

Oxidation of $H_2S$ yields $SO_4^{2-}$, electrons, and hydrogen ions (protons) as in the half-cell reactions depicted in Table 9.5. Water contributes O during formation of $SO_4^{2-}$. Electrons are passed down the respiratory chain from S to reduce $O_2$ to water and generate proton motive force for synthesis of ATP (Eq. [6], Table 9.5) as per Figs. 9.4 and 9.5. Consequently water is both used and formed in such oxidations and there may be no change in total water. Oxidation of $S^0$ to $SO_4^{2-}$ yields only 6$e^-$ per mole of S (Table 9.6, Eq. [8]) and consequently has a lower energy yield (Eq. [9]) than does oxidation of $H_2S$ to $SO_4^{2-}$ (Table 9.5, Eq. [7]). The overall reaction shows the correct stoichiometry between S, O, and H together with generation of acidity.

TABLE 9.5 Oxidation of $H_2S$ and Role of $O_2$ as Terminal Electron Acceptor by Chemolithotrophs such as *Thiobacillus* spp.

| | | | | |
|---|---|---|---|---|
| | $H_2S + 4HOH$ | → | $SO_4^{2-} + 8e^- + 10H^+$ | |
| | $8e^- + 8H^+ + 2O_2$ | → | $4HOH - 794.5$ kJ | [6] |
| Overall: | $H_2S + 2O_2$ | → | $SO_4^{2-} + 2H^+ - 794.5$ kJ | [7] |

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TABLE 9.6 $S^0$ Oxidation for Energy Yield Using $O_2$ as Terminal Electron Acceptor, in Combination with S Oxidation to Reduce $CO_2$ for Biomass Formation in Autotrophic Chemolithotrophs such as *Thiobacillus* spp.

| | | | | |
|---|---|---|---|---|
| Energy: | $S^0 + 4HOH$ | → | $SO_4^{2-} + 6e^- + 8H^+$ | [8] |
| | $6e^- + 6H^+ + 1.5O_2$ | → | $3\ HOH - 584.9$ kJ | [9] |
| | $S^0 + HOH + 1.5O_2$ | → | $SO_4^{2-} + 2H^+ - 584.9$ kJ | [10] |
| C reduction: | $4S^0 + 16HOH$ | → | $4SO_4^{2-} + 24e^- + 32H^+$ | |
| | $6CO_2 + 24e^- + 24H^+$ | → | $6CH_2O + 6HOH$ | |
| | $4S^0 + 6CO_2 + 10HOH$ | → | $4SO_4^{2-} + 6CH_2O + 8H^+$ | [11] |

(Reproduced from McGill (1996), with permission from SBCS and SLCS.)

In such autotrophic (obtain C from $CO_2$) chemolithotrophs (energy from a chemical source and electrons from a mineral source), $CO_2$ must be reduced to form biomass, represented here as $CH_2O$. Electrons for reduction of $CO_2$ are provided by the oxidation of S (Table 9.6; using $S^0$ as an example). Consequently additional S must be oxidized to provide electrons to reduce $CO_2$ [11]. Reverse $e^-$ transport reduces $NAD^+$ to NADH for subsequent reduction of $NADP^+$, probably by transhydrogenation, to NADPH (Nicholls and Ferguson, 1992), which is used in the Calvin cycle to reduce C. These reactions generate acidity in soil environments. For example, the combined set of reactions (Eq. [11]) releases 2 mol of $H^+$ per mole of $S^0$ oxidized during the processes of energy generation and of biomass formation. See Chap. 15 for further treatment of this. Such acidity influences soil pH and mineral weathering. By promoting mineral weathering, acidification during chemolithotrophic oxidation of N or S may also have influenced the course of planetary development through the formation of pedogenic clay (Kennedy *et al.*, 2006).

Large quantities of S may be oxidized to generate ATP for growth and reducing equivalents for the Calvin cycle. A way to relate S oxidation to ATP formation would add precision to understanding about soil microbial ecosystems. ATP yield can be estimated roughly from free energy change. Because of the nonstandard condition of the cytoplasm, free energy change in organisms is less than the "standard" free energy change. ATP synthesis under "cytoplasmic" conditions captures only about 40% of the "standard" free energy change (Nicholls and Ferguson, 1992). Consequently, although ATP hydrolysis yields a standard free energy change of 30 kJ $mol^{-1}$, a free energy change of about $30/0.4 = 75$ kJ $mol^{-1}$ will be expected to yield only 1 mol of ATP. Accepting this estimate, calculating the expected ATP yield using Eq. [10] yields $584.9/75 = 7.8$ mol. The Calvin cycle requires 3 mol of ATP per mole of C fixed. Rounding to 8 mol of ATP per mole $S^0$ oxidized (ATP/ $2e^- = 2.7$) and 3 mol ATP used per mole of C fixed, one can write an overall summary of the amount of S oxidized both to generate ATP and to provide the reducing equivalents needed to reduce $CO_2$ to $CH_2O$ (Table 9.7, Eq. [12]).

## Oxidation of N with Reduction of $CO_2$

Nitrogen is an important agronomic, ecological, and environmental element that is oxidized by autotrophic chemolithotrophs during nitrification. The N atoms in $NH_4^+$ are oxidized to release protons and electrons; and water contributes O for

TABLE 9.7 Estimate of Overall Stoichiometry of S Oxidation for Energy Production and C Reduction by Species such as *Thiobacillus* spp.

| | | | | |
|---|---|---|---|---|
| | $72(ADP + P_i) + 9S^0 + 9HOH + 13.5O_2$ | $\rightarrow$ | $9SO_4^{2-} + 18H^+ + 72ATP$ | |
| C reduction: | $16S^0 + 64HOH$ | $\rightarrow$ | $16SO_4^{2-} + 96e^- + 128H^+$ | |
| | $72ATP + 24CO_2 + 96e^- + 96H^+$ | $\rightarrow$ | $24CH_2O + 24HOH + 72(ADP + P_i)$ | |
| Overall: | $25S^0 + 24CO_2 + 49HOH + 13.5O_2$ | $\rightarrow$ | $25SO_4^{2-} + 24CH_2O + 50H^+$ | [12] |

(Reproduced from McGill (1996), with permission from SBCS and SLCS.)

formation of $NO_2^-$ (Table 9.8). The $O_2$ is the terminal electron acceptor and reacts with electrons and $H^+$ to form water [13]. Transport of the electrons and $H^+$ down the respiratory chain for final reaction with $O_2$ to form water generates the proton motive force used to synthesize ATP. The $H^+$ in excess of that used in reacting with $O_2$ and electrons to form water is released to the environment, causing acidification [14]. Reverse $e^-$ transport is used to transfer $e^-$ from $NH_4^+$ to NADH as in $S^0$ oxidation. Large amounts of $NH_4^+$ must be oxidized, and large amounts of $H^+$ are released into the environment during N oxidation from $NH_4^+$ to $NO_3^-$ (nitrification) to reduce $CO_2$ for $CH_2O$ production [15]. In contrast, $H^+$ is not released during oxidation of $NO_2^-$ to $NO_3^-$ (Table 9.9, Eq. [16]). Consequently one should not conclude that all chemolithotrophic oxidation reactions release $H^+$ into the soil environment.

As with S oxidation, it is instructive to estimate how much N must be oxidized to provide energy to synthesize microbial biomass of nitrifiers. To this end the quantities of N that would be oxidized for combined energy and C reduction by *Nitrosomonas* spp. are estimated in Table 9.10 and for *Nitrobacter* spp. in Table 9.11. From Eq. [14] there could be 270.7/75 = 3.6 mol ATP $mol^{-1}$ of $NH_4^+$ oxidized (ATP/2$e^-$ = 1.2). The net estimate is that 12 mol of $H^+$ are released, and 4 mol of C are reduced for every 6 mol of $NH_4^+$ oxidized to $NO_2^-$ (Eq. [18]). This $NO_2^-$ rarely accumulates in soil; it is further oxidized to $NO_3^-$ (Eq. [19]). Less energy is obtained by oxidizing a mole of $NO_2^-$ (Eq. [16]) than is obtained by oxidizing a mole of $NH_4^+$ (Eq. [14]). This partly reflects the smaller number of

**TABLE 9.8** $NH_4^+$ Oxidation both for Energy and for Reduction of $CO_2$ in Autotrophic Chemolithotrophs such as *Nitrosomonas* spp.

| | | | | |
|---|---|---|---|---|
| Energy: | $NH_4^+ + 2HOH$ | → | $NO_2^- + 6e^- + 8H^+$ | |
| | $6e^- + 6H^+ + 1.5O_2$ | → | $3HOH - 270.7$ kJ | [13] |
| | $NH_4^+ + 1.5O_2$ | → | $NO_2^- + HOH + 2H^+ - 270.7$ kJ | [14] |
| C reduction: | $4NH_4^+ + 8HOH$ | → | $4NO_2^- + 24e^- + 32H^+$ | |
| | $6CO_2 + 24e^- + 24H^+$ | → | $6CH_2O + 6HOH$ | |
| | $4NH_4^+ + 6CO_2 + 2HOH$ | → | $4NO_2^- + 6CH_2O + 8H^+$ | [15] |

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**TABLE 9.9** Oxidation of $NO_2^-$ (Nitrite) for Energy Yield Using $O^2$ as Terminal Electron Acceptor, in Combination with $NO_2^-$−N Oxidation to Reduce $CO_2$ for Biomass Formation in Autotrophic Chemolithotrophs such as *Nitrobacter* spp.

| | | | | |
|---|---|---|---|---|
| Energy: | $NO_2^- + HOH$ | → | $NO_3^- + 2e^- + 2H^+$ | |
| | $2e^- + 2H^+ + 0.5O_2$ | → | $HOH - 77.4$ kJ | |
| | $NO_2^- + 0.5O^2$ | → | $NO_3^- - 77.4$ kJ | [16] |
| C reduction: | $2NO_2^- + 2HOH$ | → | $2NO_3^- + 4e^- + 4H^+$ | |
| | $ATP + CO_2 + 4e^- + 4H^+$ | → | $CH_2O + HOH$ | |
| | $ATP + 2NO_2^- + CO_2 + HOH$ | → | $2NO_3^- + CH_2O$ | |
| | $3NO_2^- + CO_2 + 0.5O_2 + HOH$ | → | $3NO_3^- + CH_2O$ | [17] |

(Reproduced from McGill (1996), with permission from SBCS and SLCS.)

electrons released in Eq. [16] (2 $mol^{-1}$ of N) than in Eq. [14] (6 $mol^{-1}$ of N). Because $NO_2^-$ oxidation yields little energy, the ratio of $NO_2^-$ oxidized/C fixed is 5:1 (Eq. [19]), whereas it is only 1.5:1 for $NH_4^+$ oxidation (Eq. [18]) and the corresponding ratio for $S^0$ oxidation is 1.04:1 (Eq. [12]). Comparing S oxidation with N oxidation, it is also seen that more energy is released during oxidation of $S^0$ to $SO_4^{2-}$ than in oxidizing $NH_4^+$ to $NO_2^-$. This is not due to the different number of electrons involved, because each oxidation removes $6e^-$ from the element donating them. The difference here lies in the free energy change and associated potential developed for each redox couple and the corresponding difference in location at which the $e^-$ and $H^+$ enter the respiratory chain.

## OXIDATION OF REDUCED C

Aerobic oxidation of $CH_2O$ to $CO_2$ is an important source of energy for chemoorganotrophs. In its simplest form this process is the reverse of the overall oxygenic photosynthesis reaction (Table 9.12, Eq. [20]). This group of transformations

TABLE 9.10 Estimate of Overall Stoichiometry of $NH_4^+$ Oxidation for Energy Production and C Reduction by Species such as *Nitrosomonas* spp.

| | | | | |
|---|---|---|---|---|
| | $36(ADP + P_i) + 10NH_4^+ + 15O_2$ | $\rightarrow$ | $10NO_2^- + 10HOH + 20H^+ + 36ATP$ | |
| C reduction: | $8NH_4^+ + 16HOH$ | $\rightarrow$ | $8NO_2^- + 48e^- + 64H^+$ | |
| | $36ATP + 12CO_2 + 48e^- + 48H^+$ | $\rightarrow$ | $12CH_2O + 12HOH + 36(ADP + P_i)$ | |
| Overall: | $18NH_4^+ + 12CO_2 + 15O_2$ | $\rightarrow$ | $18NO_2^- + 12CH_2O + 6HOH + 36H^+$ | |
| Which can be divided by 3: | $6NH_4^+ + 4CO_2 + 5O_2$ | $\rightarrow$ | $6NO_2^- + 4CH_2O + 2HOH + 12H^+$ | [18] |

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TABLE 9.11 Estimate of Overall Stoichiometry of $NO_2^-$ Oxidation for Energy Production and C Reduction by Species such as *Nitrobacter* spp.

| | | | | |
|---|---|---|---|---|
| | $3(ADP + P_i) + 3NO_2^- + 1.5O_2$ | $\rightarrow$ | $3NO_3^- + 3ATP$ | |
| C reduction: | $2NO_2^- + 2HOH$ | $\rightarrow$ | $2NO_3^- + 4e^- + 4H^+$ | |
| | $3ATP + CO_2 + 4e^- + 4H^+$ | $\rightarrow$ | $CH_2O + HOH + 3(ADP + P_i)$ | |
| Overall: | $5NO_2^- + CO_2 + 1.5O_2 + HOH$ | $\rightarrow$ | $5NO_3^- + CH_2O$ | [19] |

(Reproduced from McGill (1996), with permission from SBCS and SLCS.)

TABLE 9.12 Oxidation of Reduced C (Represented as $CH_2O$) using $O_2$ as Terminal Electron Acceptor

| | | | | |
|---|---|---|---|---|
| | $CH_2O + HOH$ | $\rightarrow$ | $CO_2 + 4e^- + 4H^+$ | |
| | $O_2 + 4e^- + 4H^+$ | $\rightarrow$ | $2HOH$ + energy | |
| Overall: | $CH_2O + O_2$ | $\rightarrow$ | $CO_2 + HOH$ + energy | [20] |

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TABLE 9.13 Oxidation of N-Containing Organic Molecules to $CO_2$ as a Way to Obtain Energy

| | | | | |
|---|---|---|---|---|
| | $2(C_5NH_8O_4) + 2HOH$ | $\rightarrow$ | $2NH_3 + 2(C_5H_4O_5) + 6H^+ + 6e^-$ | |
| | $2(C_5H_4O_5) + 10HOH$ | $\rightarrow$ | $10CO_2 + 28H^+ + 28e^-$ | |
| | $34H^+ + 34e^- + 8.5O_2$ | $\rightarrow$ | $17HOH$ + energy | |
| Overall: | $2(C_5NH_8O_4) + 8.5O_2$ | $\rightarrow$ | $2NH_3 + 10CO_2 + 5HOH$ + energy | [21] |

L-Glutamic acid oxidation via α-ketoglutaric acid is used as an example.
(Reproduced from McGill (1996), with permission from SBCS and SLCS.)

also includes oxidation of N- or S-containing organic molecules as a source of energy. Oxidation of L-glutamic acid is one example. Such oxidations to yield energy are responsible for mineralization reactions that release plant nutrients such as $NH_4^+$ (Table 9.13). In Table 9.12, oxidation of 1 mol of C as $CH_2O$ generates $4e^-$, whereas in Table 9.13, oxidation of 1 mol of C as L-glutamic acid generates only $3.4e^-$. Consequently the amount of ATP synthesized from the oxidation of 1 mol of C from L-glutamate would be expected to be less than during the oxidation of 1 mol of C from glucose. Such N-containing energy sources are less energetically favorable than pure carbohydrates or lipids. In turn amino acids are best reserved for protein biosynthesis. The search for energy at the expense of N-containing organic molecules (Eq. [21]) in the absence of more energetically favorable alternatives is the basis for much of the N supply to crops from decomposition of humus, plant residues, and manures.

A wide array of substrates is metabolized under anaerobic conditions: sugars; organic, fatty, and amino acids; purine and pyrimidine bases; heterocyclic compounds; and polymers such as polysaccharides, proteins, and lipids. Lignin and saturated hydrocarbons are more recalcitrant under anaerobic conditions but ring oxygenation can be achieved by some anaerobes using O from HOH. Under anaerobic conditions, mechanisms to generate energy vary from use of external electron acceptors other than $O_2$ to using no external electron acceptors.

Use of oxidized minerals as alternate external electron acceptors leads to reduction of oxidized elements. $O_2$ prevents reduction of $NO_3^-$, $SO_4^{2-}$, and $N_2$; however, it is a major product from oxygenic photosynthesis. Hence environments must exist from which $O_2$ is excluded or in which it is consumed faster than it can diffuse into them. Soils are uniquely suited to provide such microsites within aggregates even under well-aerated conditions (see Chaps. 2 and 8). Consequently, anaerobic–aerobic environments must function in sequence forming a syntrophic system. A syntrophic system may be defined as one in which two or more species of organisms with contrasting characteristics require each other in order to function or survive. For example, one species may produce a product or environmental condition that is essential for a second species, which in turn produces a product or condition essential for the first. In the case of the above soil situation, the anaerobic portion of the environment and the organisms in it generates reduced minerals from oxidized forms; the aerobic portion generates $O_2$ and

**TABLE 9.14** C Oxidation Using $NO_3^-$ or $SO_4^{2-}$ as Terminal Electron Acceptor

| | | | | |
|---|---|---|---|---|
| | $NO_3^-$ accepts $e^-$ | | | |
| | $5CH_2O + 5HOH$ | $\rightarrow$ | $5CO_2 + 20H^+ + 20e^-$ | |
| | $4NO_3^- + 20H^+ + 20e^- + 4H^+$ | $\rightarrow$ | $2N_2 + 12HOH + \text{energy}$ | |
| Overall: | $5CH_2O + 4NO_3^- + 4H^+$ | $\rightarrow$ | $5CO_2 + 2N_2 + 7HOH + \text{energy}$ | [22] |
| | $SO_4^{2-}$ accepts $e^-$ | | | |
| | $2CH_2O + 2HOH$ | $\rightarrow$ | $2CO_2 + 8H^+ + 8e^-$ | |
| | $SO_4^{2-} + 8H^+ + 8e^- + 2H^+$ | $\rightarrow$ | $H_2S + 4HOH + \text{energy}$ | |
| Overall: | $2CH_2O + SO_4^{2-} + 2H^+$ | $\rightarrow$ | $2CO_2 + H_2S + 2HOH + \text{energy}$ | [23] |

(Reproduced from McGill (1996), with permission from SBCS and SLCS.)

oxidized minerals from reduced forms. The sequence of aerobic–anaerobic environments may be organized either temporally or spatially. Oxidized products are typical of the oxygenic cycle. Without a way to reduce such materials, the system would stop. The anaerobic phase, which shares elements with the anoxygenic cycle, reduces oxidized minerals to allow completion of the cycle (Table 9.14). A vast array of elements is reduced this way (Ehrlich, 1993) with N as $NO_3^-$, S as $SO_4^{2-}$, and C as $CO_2$ as frequent examples.

Reduction of oxidized forms of elements serves diverse purposes designated by specific terms. For example $NO_3^-$ may be reduced for two distinctly different reasons. The first is for incorporation into monomers such as amino acids and assimilation into polymers such as protein, which is called assimilatory $NO_3^-$ reduction. The second is to use $NO_3^-$ as a terminal electron acceptor in electron transport phosphorylation, which is termed dissimilatory $NO_3^-$ reduction because the N is not assimilated. Alternatively it is called nitrate respiration because of its parallel to oxygen respiration. When dissimilatory $NO_3^-$ reduction leads to $N_2$ production it is called denitrification (see Chap. 13 for further coverage of denitrification). When it leads to $NH_3$, it is called dissimilatory $NO_3^-$ reduction to $NH_3$. Similar distinctions apply to reduction of oxidized forms of S, such that assimilatory S reduction leads to incorporation of S into amino acids or other monomeric building blocks and eventually into biomass. On the other hand, S may be reduced as a terminal electron acceptor during oxidation of C, in which case it is called dissimilatory S reduction.

Two important distinctions exist between organisms responsible for oxidizing reduced C using $NO_3^-$ as a terminal electron acceptor (Table 9.14, Eq. [22]) and those that oxidize C using $SO_4^{2-}$ as an electron acceptor (termed dissimilatory $SO_4^{2-}$ reduction or sometimes $SO_4^{2-}$ respiration; Eq. [23]). First, $NO_3^-$ respiration is mediated by facultative anaerobes (e.g., *Pseudomonas denitrificans*), whereas $SO_4^{2-}$ respiration is mediated by strict anaerobes (e.g., *Desulfovibrio desulfuricans*). Second, the free energy change and ATP/2$e^-$ ratio is greater for $NO_3^-$ respiration than for $SO_4^{2-}$ respiration (Gottschalk, 1986). Because of these differences some people prefer to restrict the term anaerobic respiration to denitrification. This metabolic distinction and the associated greater energy yield from $NO_3^-$ respiration (Eq. [22])

compared to dissimilatory $SO_4^{2-}$ reduction (Eq. [23]) suggest that one would not expect to find dissimilatory $SO_4^{2-}$ reduction in a soil well supplied with $NO_3^-$.

The elemental cycling implications should be noted. For example, production of 1 mol of $NO_3^-$ from $NH_4^+$ consumes 2 mol of $O_2$. Subsequent reduction of that mole of $NO_3^-$ back to $NH_4^+$ will support as much oxidation of C as would the original 2 mol of $O_2$. This is because O accepts 8 mol of electrons from a mole of N and in turn a mole of N gains 8 mol of electrons from C. Similarly using $O_2$ to oxidize $S^{2-}$ to $SO_4^{2-}$ and then using $SO_4^{2-}$ to oxidize C yields the same amount of $CO_2$ as if the C had been oxidized directly with $O_2$. So where is the distinction between anaerobic oxidation by anaerobic chemoorganotrophs using $NO_3^-$ or $SO_4^{2-}$ and aerobic chemoorganotrophs using $O_2$? The difference is in the yield of ATP and hence potential for growth of biomass, which subsequently controls the rate of the process. The ATP yield is much lower under $NO_3^-$ respiration or $SO_4^{2-}$ respiration (for those who accept that term for S reduction during C oxidation) than when using $O_2$.

What about the $NO_3^-$ or $SO_4^{2-}$ generated during reduction of $CO_2$ to $CH_2O$ for growth? The treatment above considers only the production of $NO_3^-$ or $SO_4^{2-}$ associated with $O_2$ reduction. But more biogenic $NO_3^-$ and $SO_4^{2-}$ are present in ecosystems than are generated by $O_2$ reduction. For example, from Eq. [12] about 1.8 times as much $SO_4^{2-}$ is generated in reducing C with $S^0$ as is used to generate energy by reducing $O_2$. Similarly, from Tables 9.10 and 9.11, about 42% of the $NO_3^-$ generated from $NH_4^+$ originates through production of reducing equivalents to reduce $CO_2$ to $CH_2O$. Consequently the potential for oxidation of C using $NO_3^-$ or $SO_4^{2-}$ in ecosystems is greater than the consumption of $O_2$ for their production would indicate.

From a practical environmental perspective, dissimilatory $NO_3^-$ reduction, or the organisms involved in it, may be helpful in facilitating oxidation of persistent organic pollutants under anaerobic conditions. For example, Fig. 9.6 shows the oxidation of aniline by mixed cultures including denitrifiers and/or methanogens under anaerobic conditions. The initial attack is by $CO_2$, followed by investment of ATP for attachment of coenzyme A (HSCoA) and a series of reduction steps prior to oxidations and actual ring opening by insertion of water.

Is it possible to generate ATP in the absence of external electron acceptors? To do so would require an internal electron acceptor and a net H balance of 0. Further, without an external electron acceptor, there would be no need to transport electrons through a respiratory chain and hence no opportunity for electron transport phosphorylation. Consequently ATP generation would be by substrate-level phosphorylation. Fermentation is the mechanism by which ATP is generated in organisms without access to external electron acceptors. A wide range of substrates is fermented, including carbohydrates, organic acids, amino acids, and purine and pyrimidine bases. Chemically fermentation can be treated as a disproportionation reaction (sometimes called dismutation; an oxidation–reduction reaction in which a reactant or element is both oxidized and reduced leading to two different products) in which both the source of $e^-$ and the $e^-$ acceptor is an organic molecule. A divergence is seen: one product of such reactions is more oxidized than the parent molecule, and one is more reduced (Ehrlich, 1993). Ethanol

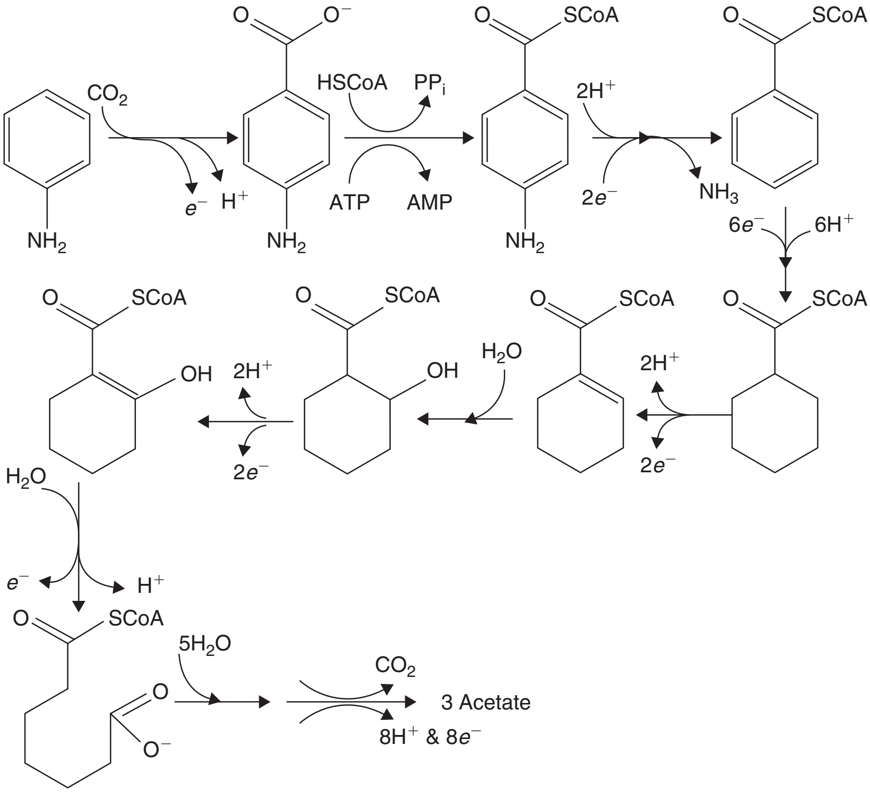



FIGURE 9.6 An example of anaerobic metabolism of aniline (denitrifiers, methanogens, or cocultures) (from Schink *et al.*, 1992, with permission from Wiley-VCH).

TABLE 9.15 An Example of Fermentation: Anaerobic Oxidation of Glucose to Form Ethanol and ATP[a]

| | | | | |
|---|---|---|---|---|
| | $C_6H_{12}O_6 + 2ATP$ | → | $2C_3H_5O_3\text{-}PO_3H_2 + 2ADP$ | |
| | $2C_3H_5O_3\text{-}PO_3H_2 + 2NAD^+ + 2H_3PO_4$ | → | $2C_3H_4O_4\text{-}2(PO_3H_2) + 2NADH + 2H^+$ | |
| | $2C_3H_4O_4\text{-}2(PO_3H_2) + 2ADP$ | → | $2C_3H_3O_3\text{-}PO_3H_2 + 2ATP + 2HOH$ | |
| | $2C_3H_3O_3\text{-}PO_3H_2 + 2ADP$ | → | $2C_3H_4O_3 + 2ATP$ | |
| | $2C_3H_4O_3 + 2NADH + 2H^+$ | → | $2C_2H_6O + 2CO_2 + 2NAD^+$ | |
| Overall: | $C_6H_{12}O_6 + 2ADP + 2H_3PO_4$ | → | $2C_2H_6O + 2CO_2 + 2ATP$ | [24] |

$C_6H_{12}O_6$, glucose; $C_3H_5O_3\text{-}PO_3H_2$, glyceraldehyde 3-phosphate; $C_3H_4O_4\text{-}2(PO_3H_2)$, 1,3-bisphosphoglycerate; $C_3H_3O_3\text{-}PO_3H_2$, phosphoenolpyruvate; $C_3H_4O_3$, pyruvae; $C_2H_6O$, ethanol.
[a]Not all intermediates are represented.

fermentation is shown in detail in Table 9.15 and several fermentation systems are summarized below (multiple arrows indicate missing steps):

Ethanol fermentation by yeasts

$$\text{Glucose} \leftrightarrow 2\text{ ethanol} + 2CO_2 + 2ATP$$

Lactic acid homofermentation by bacteria

$$\text{Glucose} \quad \leftrightarrow \quad 2\ \text{lactate} + 2\text{ATP}$$

Lactic acid heterofermentation by bacteria

$$\text{Glucose} \quad \leftrightarrow \quad \text{ethanol} + \text{lactate} + CO_2 + 1\text{ATP}$$

$H_2$ production, e.g., butyrate fermentation by bacteria

$$\text{Glucose} \quad \leftrightarrow \quad \text{butyrate} + 2CO_2 + 2H_2 + 3\text{ATP}$$

Taking ethanol fermentation as an example, the average oxidation state of the C atoms in glucose is 0 ($C_6H_{12}O_6$). Four electrons are removed from each of 2 C atoms to generate $2CO_2$ (oxidation state of C + 4), which are distributed by transferring 2 electrons to each of 4 atoms of C to form 2 ethanol molecules ($2C_2H_6O$; oxidation state of C − 2). The more reduced and the more oxidized moieties can be seen in each of the above examples except for lactic acid fermentation, in which subsequent reduction of one product may yield two identical molecules. In other cases, electrons may combine with $H^+$ to form $H_2$ using a pyruvate–ferredoxin oxidoreductase (Gottschalk, 1986) as in butyrate fermentation above.

Why bother with all this if energy as ATP is the goal? The reason is that formation of phosphorylated intermediates needed for substrate level phosphorylation requires oxidation of the organic substrate. Such oxidation removes $e^- + H^+$, which must go somewhere. With no external electron acceptor, a vast array of internal mechanisms is used to relocate the $e^- + H^+$, hence the great diversity of fermentation systems and of fermentation intermediates within anaerobic ecosystems. Extent of oxidation, energy yield, and, hence, growth under such conditions is several fold lower than under aerobic conditions.

Methanogens are the strictest anaerobes normally found in nature and use a limited array of substrates: $H_2 + CO_2$, formate, methanol, methylamines, and acetate. These substrates are formed during fermentation or converted from fermentation products in anaerobic systems. Two groups of organisms produce methane. The first is strictly chemolithotrophic organisms that grow on $H_2$ and $CO_2$. They are fascinating in their ability to produce all their needs for energy and C from $H_2$ and $CO_2$ alone. Clearly some of the reducing equivalents from the $H_2$ are used for $CO_2$ reduction as well as for energy generation. The reaction is $CO_2 + H_2 \rightarrow CH_4 + HOH$. The free energy change is −136 kJ per reaction (Gottschalk, 1986). $H_2$ can be produced by reactions such as fermentation of butyric acid to produce acetate according to $C_4H_8O_2 + 2HOH \rightarrow 2C_2H_4O_2 + 2H_2$. This is an endothermic reaction, but with a free energy change of +48.1 kJ per reaction under standard conditions (Gottschalk, 1986). Such a thermodynamically unfavorable free energy change would suggest that $H_2$ could not be released. Ecosystems have adapted, however. Thermodynamically the actual free energy change is related to the difference between equilibrium concentration and the

current concentration. Rapid utilization of $H_2$ by methanogens (among others) in close proximity to $H_2$ producers keeps the $H_2$ concentration exceedingly low compared to the equilibrium concentration thereby driving the above reaction to the right. The net free energy change for the combined reactions is negative and hence thermodynamically favorable. Hence, syntrophic associations of organisms are important in soils. The second group of organisms that produces methane is chemoorganotrophic; they produce $CH_4$ from substrates such as methanol, acetate, or methylamines, which contain methyl groups. For methanol fermentation to $CH_4$ the overall reaction is $4CH_3OH \rightarrow 3CH_4 + CO_2 + 2HOH$. Acetate is the most common and important, and its conversion to methane is written simply as $C_2H_4O_2 \rightarrow CH_4 + CO_2$. In the presence of $SO_4^{2-}$ acetate is oxidized to $CO_2$ rather than being split to $CH_4$ and $CO_2$. Here we see the preference of $SO_4^{2-}$ as a terminal electron acceptor over methane fermentation. The arrangement of anaerobic and aerobic microsites close to each other in soils facilitates commensalistic associations that favor the use of fermentation products either in methanogenesis or by aerobic or anaerobic chemoorganotrophs.

## HOW CAN THE MICROBIAL CONTRIBUTIONS BE VIEWED IN A SIMPLIFIED AND UNIFIED CONCEPT?

Two principles are fundamental to this simplified concept. They are:

1. The mechanisms by which soil organisms achieve their major functions are centered on the supply and interconversions of diverse forms of energy.
2. These mechanisms are also responsible for most other biological transformations observed in soils.

Transformations mediated by soil organisms result from their search for energy. Energy for soil organisms is obtained by passing electrons from $e^-$ donors to $e^-$ acceptors to produce ATP. Flow of $e^-$ between donors and receptors changes the oxidation states of elements. These donors and acceptors form multiple interconnected oxidation–reduction couples, which lead to cycles through which electrons flow. Because of the central role of oxidation–reduction reactions, $O_2$ availability would be expected to be, and is, is a major control on how these interconnected oxidation–reduction couples operate. Electron donors and acceptors in each couple are often different elements. Consequently these flows of $e^-$ unite cycles of elements, alter mobility and functions of elements, and regulate soil biological transformations. Flow of electrons among these cycles unites activities of extremely diverse groups of soil organisms. Therefore, one can understand most soil biological transformations as a simple framework of interconnected cycles of $e^-$.

Such a conceptualization is a simple and robust way to unite the myriad details about transformations mediated by soil organisms. An electron cycle model

accommodates the full spectrum of understanding from detailed presentations of reactions to global biogeochemical cycles and earth history. It further makes predictions about what we might yet find in nature concerning mineral transformations by soil organisms.

## A MODEL OF INTERCONNECTED CYCLES OF ELECTRONS

This system of flowing $e^-$ is like an electrical system with two cycles, an anoxygenic (not $O_2$-producing) cycle and an oxygenic ($O_2$-producing) cycle, and four circuits, two phototrophic, one chemoorganotrophic, and one chemolithotrophic, hooked in parallel (Fig. 9.7). The microbial component consists of four groups of organisms described using the nutritional classification in Table 9.1. Three groups of organisms are responsible for C addition to the pedosphere, and only one (chemoorganotrophs) for its removal. The two cycles are distinguished on the basis of the photosynthetic mechanisms: anoxygenic or oxygenic. The chemoorganotrophic circuit unites the anoxygenic cycle with the oxygenic cycle.

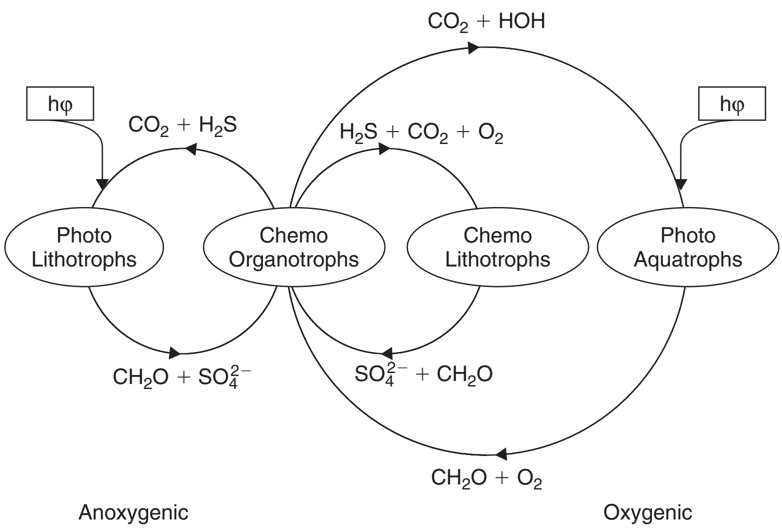



FIGURE 9.7 Schematic outline of electron flow through oxygenic and anoxygenic cycles starting with anoxygenic (not oxygen-producing) on the left and oxygenic photosynthesis (oxygen-producing) on the right. Anoxygenic photosynthesis (photolithotrophs) generates oxidized S species from reduced S under anaerobic conditions, thereby maintaining anaerobic conditions. Oxygenic photosynthesis (photoaquatrophs) generates oxygen from water, thereby producing aerobic conditions. Both yield $CH_2O$ for biomass production. In contrast, however, photolithotrophs serve the mineral-oxidizing function of chemolithotrophs in oxygenic cycles, but do not produce oxygen. Chemoorganotrophs unite both sets of cycles by oxidizing reduced C aerobically or anaerobically to $CO_2$. (Reproduced from McGill (1996), with permission from SBCS and SLCS.)

## THE ANOXYGENIC CYCLE

Anoxygenic photosynthesis uses energy from sunlight to couple the reduction of C in $CO_2$ to the anaerobic oxidation of S in $S^0$ or $H_2S$. If one is accustomed to thinking of S oxidation in a strictly aerobic sense, then anaerobic S oxidation appears contradictory. Anoxygenic photosynthesis would have been compatible with the anoxic ($O_2$-free) conditions of the earth's primordial atmosphere. It could have been mediated by anaerobic organisms like present-day photosynthetic sulfur bacteria and is believed to have preceded oxygenic photosynthesis (Staley and Orians, 1992). Dominance of anoxygenic photosynthesis would have favored anaerobic respiration or fermentative pathways for obtaining energy from the products of photosynthesis. Consequently one might expect, and certainly one finds, a wide representation of anaerobic microorganisms in soil environments. Many elements can cycle under entirely anaerobic conditions due to the syntrophic relationships among photolithotrophs and anaerobic chemoorganotrophs. With the input of electromagnetic radiation to power photosynthesis by photolithotrophs that reduce C and oxidize elements such as S, combined with decomposition by anaerobic chemoorganotrophs to reoxidize C and re-reduce S, one can summarize the anoxygenic cycle as:

$$4CO_2 + 2H_2S + 4HOH \quad \leftrightarrow \quad 2SO_4^{2-} + 4CH_2O + 4H^+.$$

Energy trapped as organic compounds during anoxygenic photosynthesis could be released by oxidation of the reduced C ($e^-$ donor) coupled to reduction of the oxidized minerals ($e^-$ acceptor) formed during anoxygenic photosynthesis.

## THE OXYGENIC CYCLE

Oxygenic photosynthesis can be carried out by many eukaryotes, but only by the cyanobacteria among the prokaryotes. Consequently, anoxygenic photosynthesis is dominantly prokaryotic and oxygenic photosynthesis dominated by eukaryotes. The oxygenic cycle has two circuits. First, a photo–chemo circuit consisting of photoaquatrophs to reduce C coupled with chemoorganotrophs, which may be either aerobic or anaerobic, to reoxidize it. Second, a chemo–chemo circuit consisting of aerobic chemolithotrophs to oxidize minerals coupled with anaerobic (or facultative) chemoorganotrophs to oxidize C and re-reduce minerals (Fig. 9.7). Electrons flow among the cycles, thereby connecting them. For example, an electron from water may be passed to $CH_2O$ during photosynthesis, proceed through the photo–chemo circuit, and be returned to water through aerobic oxidation. From there it may again be passed to $CO_2$ (photosynthesis) to form $CH_2O$, proceed to the chemo–chemo circuit, and under anaerobic conditions be used to reduce $SO_4^{2-}$ to $H_2S$. In the presence of $O_2$, it proceeds through the aerobic part of the chemo–chemo circuit and the electron is again used to reduce $CO_2$ to $CH_2O$ concurrently with oxidation of (loss of electrons from) $S^{2-}$ As it continues its journey, under anaerobic conditions the electron may again be transferred to $H_2O$, which brings it to the intersection

again with the photo–chemo circuit. Hence, the oxidation and reduction of many elements, although not conducted by photosynthetic organisms, is tied to photosynthesis by transfers of electrons among organisms through the reduced C and $O_2$ produced by photosynthesis.

Hence, the oxidation of elements is made possible by $O_2$ from photosynthesis and their reduction by the reduced C from photosynthesis. This alternating oxidation–reduction system involving chemolithotrophs requires that $O_2$ and $CH_2O$ from photosynthesis travel separately and that there be habitats from which the $O_2$ is excluded. Soils are uniquely suited to providing such habitats.

The two chemotrophic circuits of Fig. 9.7 can be expanded to distinguish aerobic, facultative, and anaerobic domains based on sensitivity to $O_2$ (Fig. 9.8). The

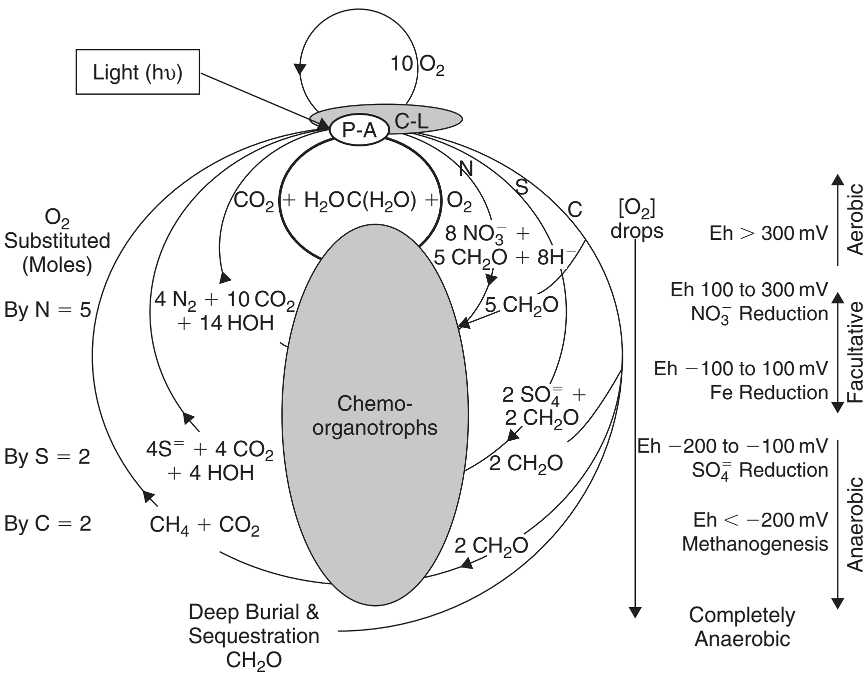



FIGURE 9.8 The oxygenic cycles in the pedosphere entail cyclic oxidations and reductions of C, N, and S (among other elements), which are driven by solar radiation and controlled by the availability of $O_2$. Lowering $O_2$ availability is reflected in lowering $E_h$ values. Oxygenic photoaquatrophs (P-A) produce $CH_2O$ and release $O_2$; aerobic chemolithotrophs (C-L) oxidize N to $NO_3^-$ or S to $SO_4^{2-}$ using $O_2$ from photosynthesis and reduce $CO_2$ to $CH_2O$ autotrophically. Aerobic chemoorganotrophs oxidize $CH_2O$ using $O_2$ through aerobic respiration (heavy circle); and facultative anaerobic chemoorganotrophs oxidize $CH_2O$ using $NO_3^-$ (nitrate respiration). Anaerobic chemoorganotrophs oxidize $CH_2O$ by reducing $SO_4^{2-}$ or, under extremely anaerobic conditions, by reducing a portion of the $CH_2O$ itself, thereby splitting it into $CO_2$ (oxidized) and $CH_4$ (more reduced). For the stoichiometry represented here the moles of $O_2$ substituted by N, S, or C are designated at the left. Dinitrogen fixation using reduced C ultimately from P-A is required to reduce $N_2$ to $NH_3$ prior to its oxidation to $NO_3^-$. Deep burial of $CH_2O$ opens the oxygenic cycles thereby allowing $O_2$ to accumulate.

aerobic domain is set at $E_h$ (oxidation–reduction potential) >300 mV (see Fig. 15.2), comprising aerobic chemoorganotrophs and photoaquatrophs as a syntrophic system. The facultative domain, between $E_h$ 100 and 300 mV, comprises chemoorganotrophs, which may be either aerobic or anaerobic in syntrophic associations with chemolithotrophs, which are aerobic. The anaerobic domain consists of strictly anaerobic chemoorganotrophs in association with photoaquatrophs. Anaerobic chemoorganotrophs in the anaerobic domain re-reduce oxidized minerals generated by aerobic chemolithotrophs in the aerobic domain. Some energy is dissipated through heat loss, etc., and must be made up by subsequent photosynthesis.

Overall control is provided by $O_2$ because it inhibits anaerobic processes. Consequently the balance among the three domains in Fig. 9.8 is a function of $O_2$ availability in local environments or microsites. $N_2$ fixation is interesting in that it is inhibited by $O_2$ and mediated by three of the four groups of organisms, with only chemolithotrophs excluded. In addition, the chemoorganotrophs are responsible both for removing N from the pedosphere, by reducing $NO_3^-$ to $N_2$, and for returning it by further reducing $N_2$ to $NH_3$.

As the $E_h$ becomes increasingly negative oxidants become decreasingly effective (Fig. 9.8). Given that the energy available through a redox reaction is directly proportional to the change in oxidation potential between the two couples, less and less energy is released as one moves from aerobic to strictly anaerobic metabolism. Soil organisms have evolved in an energy-limited environment so communities of soil organisms use the most energetically favorable energy sources available to them. Such a strategy favors use of $O_2$ as an electron acceptor followed by $NO_3^-$, then $SO_4^{2-}$; after depletion of $NO_3^-$ and $SO_4^{2-}$ a portion of the C in $CH_2O$ is eventually used during methanogenesis. In other words, $CH_2O$ is allocated first to reduction of $O_2$, then $NO_3^-$ followed by $SO_4^{2-}$, and finally methanogenesis. From a practical perspective, then $NO_3^-$ might be a useful electron acceptor for metabolism of organic contaminants under anaerobic conditions. Indeed $NO_3^-$ and denitrifying populations have been proposed for removal of organic contaminants under anaerobic conditions.

The $O_2$ produced during photosynthesis is consumed stoichiometrically during complete decomposition of photosynthate (compare Eqs. [1] and [20]). It is not possible for $O_2$ to accumulate in the atmosphere from photosynthesis unless large quantities of $CH_2O$ are not decomposed. We are not awash in nondecomposed plant litter, so how can residual $O_2$ have accumulated in the atmosphere? Disrupting the cycle of photosynthesis and decomposition would retain $O_2$ in the atmosphere rather than consuming it in oxidation of photosynthate. Disruption occurs by deep burial and sequestration of $CH_2O$ as represented in Fig. 9.8. Processes yielding soil humus, peat, shale, coal, or petroleum and deep ocean organic sediments among others over geologic time all remove $CH_2O$ from the attention of microbes that would use it to reduce $O_2$ to water (see Logan *et al.*, 1995). See Chap. 12 for further discussion on humus formation. Soil microbes may play an interesting role in preservation of reduced C over geologic time scales. For example, Kennedy *et al.* (2006) showed a strong relation between the increase of pedogenic clay and the

increases in atmospheric oxygen in the later Precambrian. They postulated that soil biological activity, including that of plant roots, weathered soil minerals, resulting in formation of pedogenic clay, which in turn was instrumental in adsorbing and sequestering C. In turn, higher $O_2$ concentrations appear to be related to development of higher animals (Kennedy *et al.*, 2006) and development of large placental mammals (Falkowski *et al.*, 2005). Might we imagine that "soil microbes rule the world"?

Given that C storage could allow $O_2$ accumulation, what might be the control on maximum $O_2$ accumulation? Mineral weathering is a sink for $O_2$ and participates in regulating atmospheric $O_2$ concentration. In addition, oxidation–reduction reactions over geological time control Fe solubility and, because of its reactivity with P, Fe controls P concentration. Fe and P concentrations in solution, especially in marine environments, often limit photosynthesis and $N_2$ fixation. Regulation of Fe and P solubility by the oxidation state of Fe provides a feedback to atmospheric $O_2$ concentration (Van Cappellen and Ingall, 1996).

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# 10

# The Ecology of Plant–Microbial Mutualisms

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## INTRODUCTION

Plants interact with a multitude of soil microorganisms in many different ways that have important consequences for plant growth and fitness. When microorganisms colonize and live within a host plant, these interactions are called "symbioses." In mutualistic symbioses, in which all partners benefit, the plant generally supplies its symbiont with photosynthetic C in exchange for a limiting resource (such as a nutrient or water) or protection from antagonists. Mycorrhizal fungi, the most ubiquitous of root-associated plant symbioses, attain photosynthetic C from the plant partner in return for enhanced nutrient and water uptake, resulting from an increased effective root surface area or increased uptake efficiency of certain nutrients. Mycorrhizal plants can have enhanced growth compared to uncolonized

plants under low nutrient concentrations and under conditions where movement of nutrients in soil is reduced (e.g., drought). Leguminous plants form rhizobial symbioses with $N_2$-fixing bacteria. Rhizobia infect the roots of the host plant, which then initiate the development of root nodules where rhizobia fix atmospheric N in exchange for photosynthetic C. Other $N_2$-fixing bacteria, such as *Frankia* spp., enter into the same type of symbiosis as rhizobia, but only with a small group of nonleguminous plant species.

In practice, however, it is not always easy to observe benefit for all partners. The outcome of the symbiosis can range from mutualism to parasitism, and this can depend on interactions with the environment along spatial and temporal scales. One reason for this apparent contradiction is that we are not fully aware of the complete spectrum of ecological and evolutionary consequences of these so-called mutualistic relationships. Plants could derive indirect benefits from their microbial partners via effects on physical and biotic soil components, interactions with pathogenic microorganisms, or mediation of competition or herbivory. The long-term benefits of the symbiosis, derived at times of stress or disturbance, may outweigh negative consequences that are transient in nature.

In this chapter, we will continue to refer to symbioses, in general, that have potential to be mutualistic (mycorrhizal, rhizobial, and actinorrhizal symbioses) as "mutualistic," to distinguish them from symbioses that are obviously parasitic (involving plant pathogens). However, when describing specific types of mutualistic symbioses, they will be referred to as "symbioses."

## ROOTS AS AN INTERFACE FOR PLANT–MICROBIAL MUTUALISMS

Mutualisms among plants and microorganisms are much more common below ground than above ground, even though diverse interactions between plants and microorganisms occur in both realms. Saprotrophs and plant–pathogenic microbes dominate in the phyllosphere and their community composition differs considerably from that of the rhizosphere (Lindow and Brandl, 2003). Mutualisms among plants and microorganisms are not entirely absent in the phylloplane but appear to be limited to a few that influence plant–herbivore interactions (e.g., Clay, 1990). Functional aspects might explain the apparent difference in the prevalence of mutualisms in the two environments. Soil is a heterogeneous environment in which mutualists aid plants in exploiting ephemeral resource patches by converting organic nutrients into usable inorganic forms and through the production of structures that extend the effective zone of water and nutrient uptake by roots. Above ground, the primary resources for which plants compete are light, pollinators, and seed dispersal agents. Some fungi manipulate plant morphology and, indirectly, pollinator behavior in order to facilitate dispersal of fungal gametes, but this interaction is detrimental to plant fitness (Roy, 1993). We are not aware of any evidence that the ability of plants to capture light is enhanced through the production of specialized structures by

phyllosphere microorganisms. Plants that form mutualistic symbioses with soil microorganisms, however, can grow larger as a result of enhanced nutrient uptake, allowing these plants to compete more vigorously for light and, possibly, achieve greater fitness.

## MYCORRHIZAL SYMBIOSES

Mycorrhizas are symbioses between nonpathogenic fungi and the roots of the host plant. The mycorrhizal symbiosis is ubiquitous, is present in nearly all plant species, and can provide mutual benefits to the participants. Mycorrhizal classification is based on anatomical features of the root–fungus interface and the taxonomic classification of the fungus and host plant (Table 10.1). Peterson *et al.* (2004) presented a detailed overview of the different mycorrhizal types and many spectacular images of plant, fungal, and mycorrhizal structures. Arbuscular mycorrhizal (AM) fungi occur in symbioses with plant taxa belonging to $>90\%$ of vascular and nonvascular plant families. The taxonomic diversity of AM fungal species is relatively low (estimates range from $\sim$150 to 200 spp.) and confined to the Phylum Glomeromycota (formerly the Order Glomales within the Zygomycota) based on 18S rRNA sequence data. Ectomycorrhizal (EM) fungi form symbioses with several gymnosperm and angiosperm species and are taxonomically diverse, with approximately 6000 species; Kendrick (2001) notes 74 genera in the Basidiomycota and 16 genera in the Ascomycota. Other types of mycorrhizas are more restricted with regard to the phylogenetic diversity of the plant species in which associations are formed. Ectendomycorrhizas can be found in roots of the conifer genera *Larix* and *Pinus.* Arbutoid and ericoid mycorrhizas occur for ericaceous plants, although the former can be found in some of the Pyrolaceae (wintergreen family) and the latter can also occur in some bryophytes (nonvascular plants such as mosses, liverworts, and hornworts). Monotropoid and orchid mycorrhizas form only in roots of the plant families Monotropaceae (e.g., Indian pipe, pinedrops) and Orchidaceae, respectively; the fungal partner, however, may be able to form other types of mycorrhizal associations with other plants. Plants that associate in monotropoid mycorrhizas are achlorophyllous and nonphotosynthetic (termed mycoheterotrophic) and rely on the fungus to form additional mycorrhizal associations, often of different types, with other plants in order to attain photosynthate. Orchid mycorrhizas function in a similar way and the presence of the fungal symbiont is required prior to seed germination and seedling establishment.

The formation of mycorrhizal symbioses is complex and involves recognition, infection, and then internal colonization of roots. The process differs depending on the type of mycorrhiza (Figs. 10.1 and 10.2). AM fungi can colonize host plants via intact mycelial networks, hyphal fragments, or individual spores. Root exudates stimulate hyphal branching and directed growth. Once a hypha contacts a root, the fungus forms an appressorium from which it attempts infection. If successful, hyphae penetrate epidermal cells, enter the cortex, and grow intercellularly (or intracellularly

**TABLE 10.1** The Classification of Mycorrhizal Associations Based on Fungal Morphology (Modified from Dalpé, 2003)

| | Arbuscular | | Ecto- | Ectendo- | Arbutoid | Ericoid | Monotropoid | Orchid |
|---|---|---|---|---|---|---|---|---|
| Hyphal structures | Arum type | Paris type | | | | | | |
| Vesicles | + | + | – | – | – | – | – | – |
| Arbuscules | + | – | – | – | – | – | – | – |
| Coils | – | + | – | + | + | + | – | + |
| Pegs | – | | – | – | – | – | + | – |
| Mantle | – | | + | + | + | – | + | – |
| Hartig net | – | | + | + | + | – | + | – |
| Fungal partners | Glomeromycetes | | Zygomycetes, Ascomycetes, Basidiomycetes | Ascomycetes, Basidiomycetes | Basidiomycetes | Ascomycetes | Basidiomycetes | Basidiomycetes |
| Plant partners | Herbaceous plants, Shrubs, Deciduous trees | | Conifer trees, Some shrubs and deciduous trees | Conifer trees, Some shrubs and deciduous trees | Ericales, Pyrolaceae | Ericales, Bryophytes | Monotropaceae | Orchidaceae |

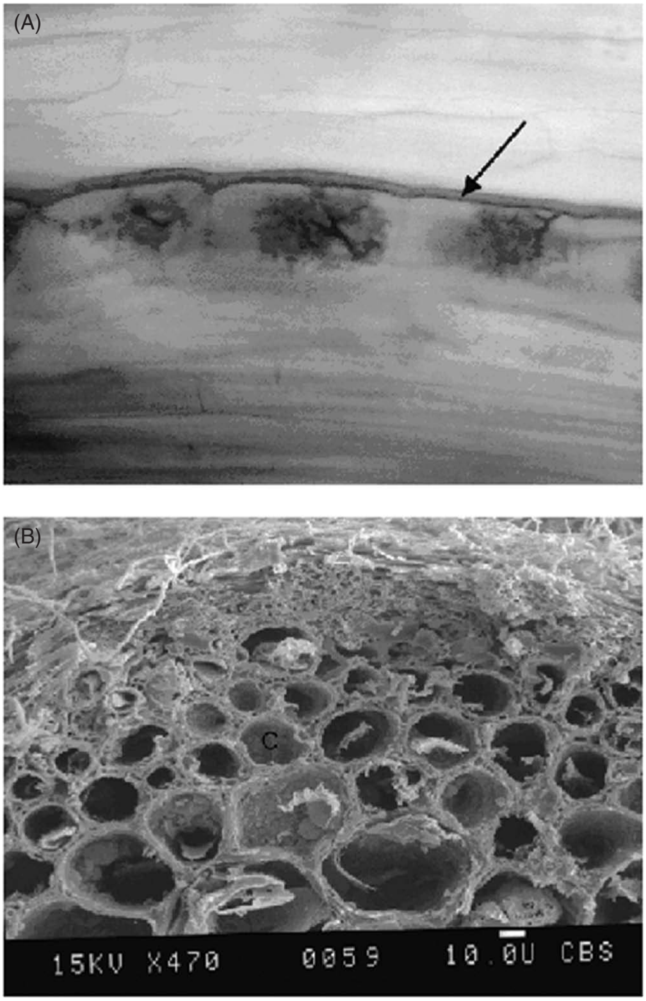



FIGURE 10.1 (A) Intercellular hyphal colonization (arrow) and intracellular arbuscular colonization of *Glycine max* roots by an arbuscular mycorrhizal fungus. (B) Mantle and Hartig net hyphae of an ectomycorrhizal fungus (*Truncocolumnella* sp.) colonizing the intercellular space between the epidermal cells (C) of a pine root. (Image courtesy of Larry Peterson.)

using hyphal coils). Finally they penetrate cortical cells and form arbuscules (or arbuscules can form directly from hyphal coils), where most nutrient exchange is believed to occur. In EM, arbutoid, and monotropoid mycorrhizas, the fungus forms a fungal mantle over the surface of the root. Hyphae penetrate the root and proliferate within the intercellular space forming a Hartig net. EM fungal colonization is limited to these intercellular spaces, while arbutoid and monotropoid fungi further penetrate cells.

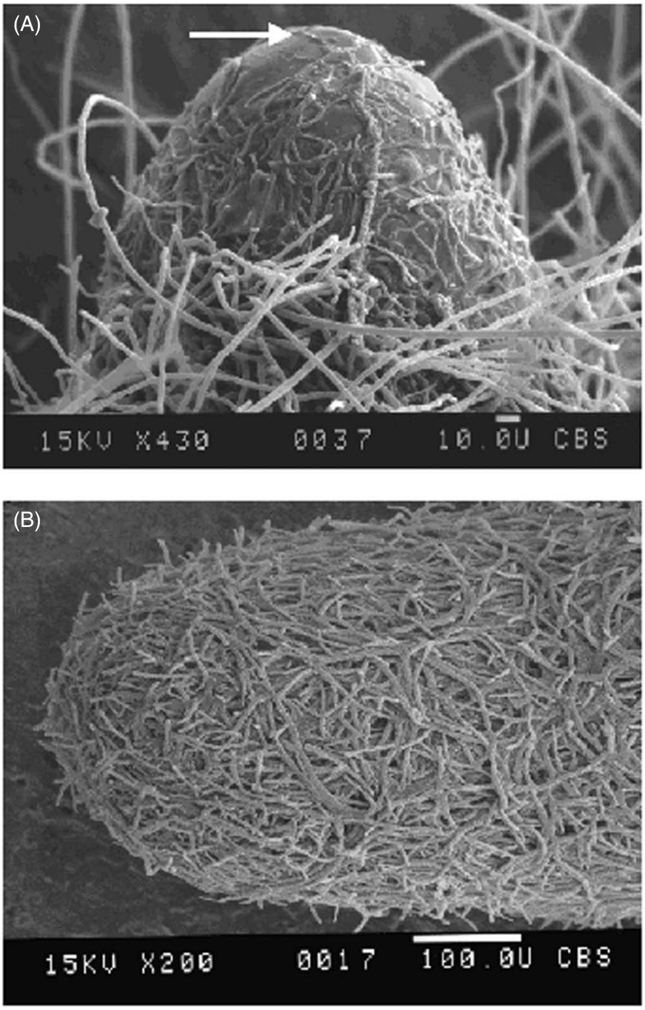



FIGURE 10.2 Colonization of pine short roots by ectomycorrhizal fungi. (A) Early stage: hyphae (*Cenococcum* sp.) are starting to proliferate on the root surface near the apex (arrow). (B) Late stage: mantle hyphae (*Rhizopogon* sp.) cover the entire root surface. (Images courtesy of Larry Peterson.)

Mycorrhizas are ancient; fossil evidence from the Devonian (400 million years ago (Mya)) indicates early land plants formed endophytic associations with fungi resembling the modern AM fungi that colonize modern bryophytes. Fossils of spores resembling AM fungi have been found in rocks from the Ordovician (460 Mya), the period during which plants first colonized land (Fig. 10.3). Early land plants resembled modern bryophytes, had poorly developed root systems, and would have benefited greatly from mycorrhizal symbioses.

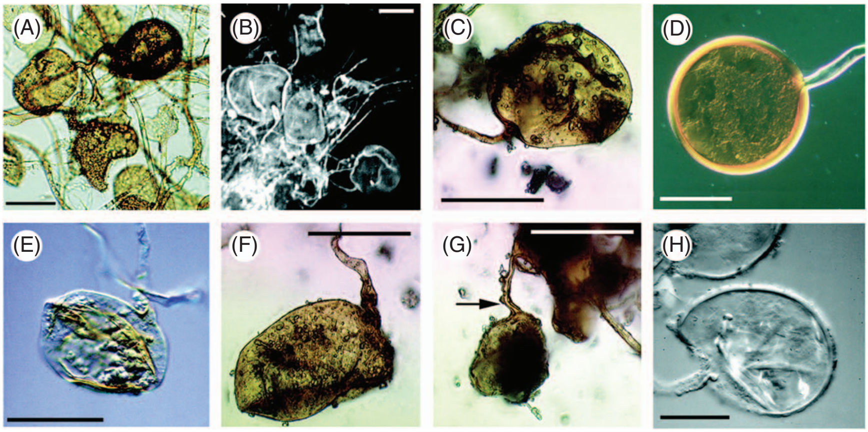



FIGURE 10.3 (A–C, E–G) Fossil spores from the Ordovician and (D, H) spores from extant arbuscular mycorrhizal fungi. Scale bars, 50 μm. (Reprinted with permission from Redecker, Kodner, and Graham, 2000; copyright 2000, AAAS.)

The primary benefit derived by plants involved in mycorrhizal symbioses is generally perceived to be enhanced nutrient uptake, achieved by the fungus expanding the zone of nutrient uptake farther away from the rhizosphere and/or more efficiently taking up and transporting nutrients. The nutrients that are taken up by the fungus depend on the type of mycorrhiza. Generally, all types of mycorrhizal fungi are able to transport N and P. Ericoid mycorrhizal fungi primarily increase the plant's access to N, but also to P, Ca, and Fe. AM fungi have been studied mostly for their ability to enhance P nutrition, but also play a role in the uptake of N, K, and Zn. EM fungi have saprotrophic abilities and are able to access organic forms of N and P. As described above, monotropoid and orchid mycorrhizal fungi transport C, in addition to N and P, to their mycoheterotrophic hosts.

However, the symbiotic role of mycorrhizal fungi is not limited to enhancing the plant's nutritional status (Fig. 10.4). Mycorrhizal fungi play an important role in the formation of soil macroaggregates, which help maintain soil stability during alternating periods of wetting and drying. Other benefits accrued by the plant may result from altered biotic interactions and take the form of defense, with mycorrhizal roots being colonized by fewer disease-causing organisms or suffering less grazing by root herbivores than nonmycorrhizal roots. The consequences of these interactions are not confined to the soil: plant fitness may be altered by mycorrhizal colonization as a result of altered interactions with insects, including herbivores, natural enemies of herbivores, and pollinators. Performance of plants and herbivores in response to mycorrhizal colonization varies depending on the feeding strategy of the herbivore and differs among fungal species (Gehring and Whitham, 2002). Parasitism of a leaf-mining insect, *Chromatomyia syngenesiae*, by a generalist parasitoid *Diglyphus isaea* was reduced on *Glomus mosseae*-colonized plants but increased on *Glomus caledonium*- and *Glomus fasciculatum*-colonized plants, possibly due

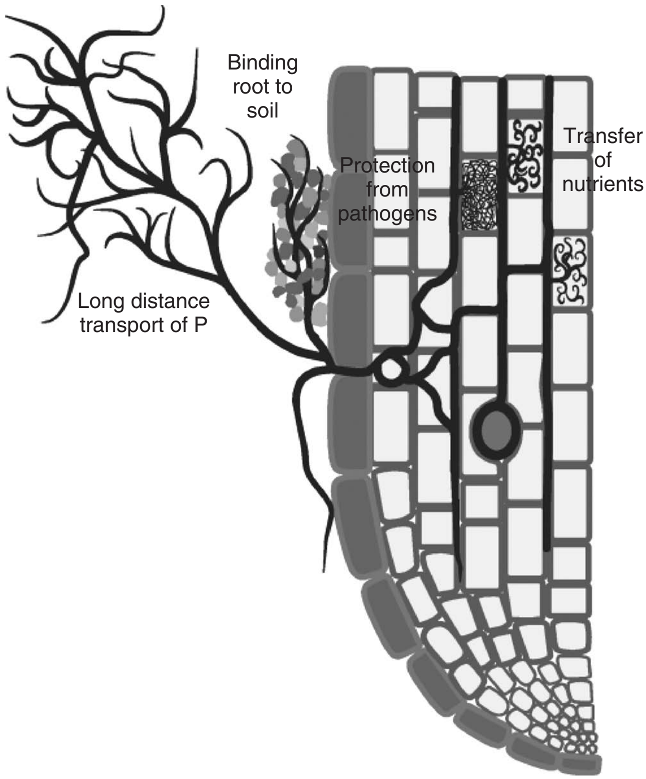



FIGURE 10.4 The multifunctional nature of AM fungi. Morphological requirements for different mycorrhizal functions include diffuse extraradical mycelium far from the root (P uptake) and in the rhizosphere (plant–soil water relations) and extensive intraradical colonization by hyphae (pathogen protection) and arbuscules (P transfer). (Reprinted from Fitter, 2005, with permission; copyright 2005, Blackwell Publishing.)

to altered host searching efficiency by the parasitoid on larger and smaller plants (Gange *et al.*, 2003). Plant–pollinator interactions may be affected by AM fungi via changes in floral morphology (Philip *et al.*, 2001) and enhanced pollen quality (Poulton *et al.*, 2001) such that pollinator visitation rates are enhanced (Wolfe *et al.*, 2005). Mycorrhizal fungi, particularly EM and/or AM fungi, also show promise for the breakdown of organic pollutants, protection of plants from metal phytotoxicity, and sequestration of toxic elements in contaminated soils.

Recently, as mycorrhizal researchers have learned more about the ecological outcomes of mycorrhizal symbioses, the concept of "mycorrhiza = mutualism" has been challenged. In orchid mycorrhizas, for example, the plant partner is achlorophyllous for an extended period following seedling emergence, thus the fungal partner receives no known benefit from the association. Achlorophyllous plants within the Monotropaceae obtain photosynthate by establishing monotropoid mycorrhizas with EM fungi already associated with a gymnosperm host; as a result,

the achlorophyllous plant effectively parasitizes the gymnosperm via the fungus, although the consequence for fungal fitness is not known. The degree to which the associates derive benefits may differ depending on environmental conditions. AM symbioses are expensive for plants to maintain, with AM fungi requiring up to 20% of host photosynthate during colonization and while functioning (Graham, 2000). Under conditions of high nutrient availability, if C costs exceed the benefits accrued by mycorrhizal uptake of nutrients, the association may actually be detrimental to the plant host. AM fungal colonization of plants is reduced under conditions of high P availability, probably due to reduced exudation of compounds stimulatory to spore germination and hyphal growth, but possibly even due to presence of plant-derived inhibitory compounds in root exudates, suggesting plant control over colonization events (Vierheilig, 2004). In addition, the species composition of an AM fungal community differs depending on soil fertility; fertilization can select for AM fungal species that promote growth to a lesser extent than those found in unfertilized soil (Johnson, 1993). The outcome of the symbiosis is also dependent on the plant and fungal species involved in the association, with effects on plant growth ranging from positive to negative across a variety of plant–fungal species combinations (Klironomos, 2003; Fig. 10.5).

Mycorrhizal symbioses are also important for shaping the environment in which plants and fungi grow. The microbial composition of mycorrhizal root systems is very different from that of nonmycorrhizal roots, a phenomenon known as the "mycorrhizosphere effect" (Linderman, 1988; Vierheilig, 2004), due to chemical and physical effects on the soil environment. Roots colonized by mycorrhizal fungi are hypothesized to have altered root membrane permeability, which may affect the abundance and composition of root exudates; few studies have measured these changes in root exudate composition, and in those studies only certain groups of identifiable compounds (e.g., phenolics) were considered. Reduced mineral and nutrient concentrations in soil, as a result of increased uptake by mycorrhizal fungi, as well as higher C inputs due to high turnover of the fungal mycelium may also contribute to changes in microbial community composition. Some mycorrhizal fungi can secrete compounds into the rhizosphere that are antagonistic to soil microorganisms; for example, *Paxillus involutus*, an EM fungus, produces ethanol-soluble substances that suppress sporulation in a pathogenic fungus, *Fusarium oxysporum* (Duchesne *et al.*, 1988).

While mycorrhizal fungi influence the composition and activity of soil microbial communities, soil microbes also reciprocate by influencing fungal activity and functioning. Microscopic examination of the surfaces of AM fungal spores and hyphae reveals colonization of surfaces by bacteria. Xavier and Germida (2003) isolated several bacterial species associated with *Glomus luteum* (formerly *Glomus clarum*) spores reared from greenhouse cultures, some of which had stimulatory or inhibitory effects on spore germination and/or hyphal growth. Both diffusible and nonvolatile substances produced by bacteria are thought to be involved in these effects on AM fungal activity. *Bacillus chitinosporus*, which inhibited spore germination in the *in vitro* bioassay, reduced *G. luteum* colonization of pea roots

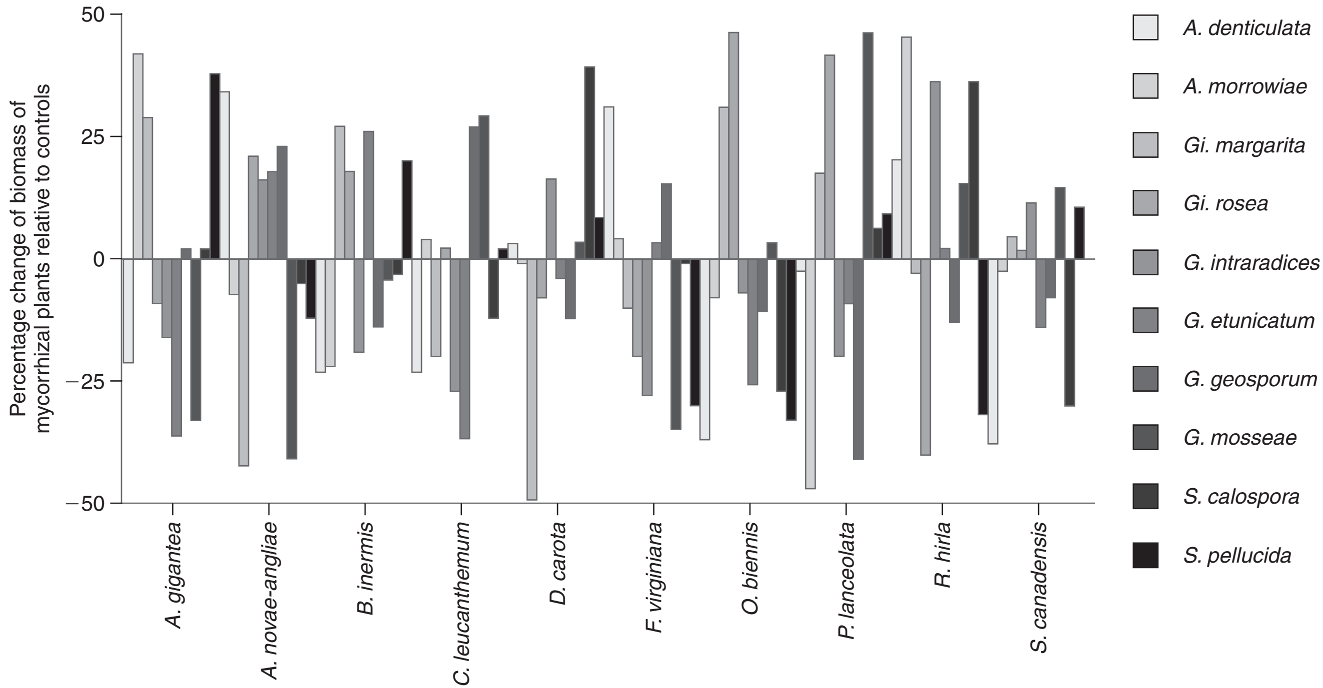



FIGURE 10.5 The influence of different species of arbuscular mycorrhizal fungi isolated from the Long-Term Mycorrhizal Research Site (LTMRS; Guelph, ON, Canada) on the growth of different plant species, relative to nonmycorrhizal controls. Plant seeds used in this experiment were also collected from the LTMRS. Plant responses to inoculation with the different fungal species ranged from highly positive to highly negative. Plant genera, from left to right along the horizontal axis, are *Agrostis*, *Aster*, *Bromus*, *Chrysanthemum*, *Daucus*, *Fragaria*, *Oenothera*, *Plantago*, *Rudbeckia*, and *Solidago*. Fungal genera, in the legend, are *A*, *Acaulospora*; *Gi*, *Gigaspora*; *G*, *Glomus*; *S*, *Scutellospora*. For all treatments, the 95% confidence interval is ±18%. (Reprinted from Klironomos, 2003, with permission; copyright 2003, Ecological Society of America.)

by 64% relative to that in the absence of *B. chitinosporus.* However, *Bacillus pabuli*, which enhanced spore germination and hyphal growth in the *in vitro* assay, had no effect on *G. luteum* colonization of pea roots. "Helper" bacteria that have stimulatory effects on hyphal growth and EM formation have been isolated from mantle hyphae, while a range of stimulatory, neutral, and beneficial effects of soil bacteria on EM colonization of plant roots has also been observed (Azcon-Aguilar and Barea, 1992).

## SYMBIOSES INVOLVING N-FIXING ORGANISMS

Leguminous plants have evolved morphologically and functionally specialized symbioses with a group of bacteria (rhizobia) in which the bacteria are rewarded with photosynthetic C in exchange for fixed N from atmospheric $N_2$. The process occurs in root nodules that form when rhizobia infect the host via root hairs and trigger changes to root morphology (Fig. 10.6). Three new genera (*Allorhizobium*, *Mesorhizobium*, and *Sinorhizobium*) have been proposed, in addition to the genera (*Azorhizobium*, *Bradyrhizobium*, and *Rhizobium*) that existed when the previous edition of this book was published, in 1996. As a result of this symbiosis, leguminous plants tend to be early successional species and good colonizers of N-poor environments. They also facilitate the establishment and growth of non-$N_2$-fixing plant species through the accumulation of fixed N in the soil environment. Several legume species are among the most widespread and abundant agricultural crops, including alfalfa, clover, beans, and soybeans. These agricultural legumes are responsible for a large portion of global biological $N_2$ fixation.

The actinorhizal symbiosis between primarily woody plants and an actinomycete, *Frankia,* is less common than the rhizobium–legume association but is important for plant species that typically colonize stressed, N-poor sites in temperate regions. Familiar host species include alder (*Alnus*), bayberry (*Myrica*), sea buckthorn (*Hippophae*), and autumn olive (*Elaeagnus*) in temperate regions and beefwoods (*Casuarina*) in the tropics. The *Frankia*–actinorhizal symbiosis differs from the legume–rhizobium symbiosis in a number of ways. For example, *Frankia* exist within nodules in a vegetative, filamentous form, while rhizobia occur as bacteroids, resembling bacteria (Fig. 10.6). Also, while the genes involved in nitrogen fixation show some sequence homology, little similarity has been found among genes involved in nodule formation (Verghese and Misra, 2002).

Like in mycorrhizal symbioses, nodulation involves the recognition and response of both partners (Chap. 14). Briefly, bacteria adhere to the surface of root hairs, which then curl around the bacteria. Bacteria then initiate an infection thread that penetrates into the cortical region of the root, stimulating meristematic growth and nodule formation. Symbioses between plants and $N_2$-fixing organisms, while morphologically and functionally less diverse than mycorrhizal associations, can be categorized by the types of nodules that are formed: determinate and indeterminate (Fig. 10.7). Rhizobia are found associated with both nodule types, but *Frankia*

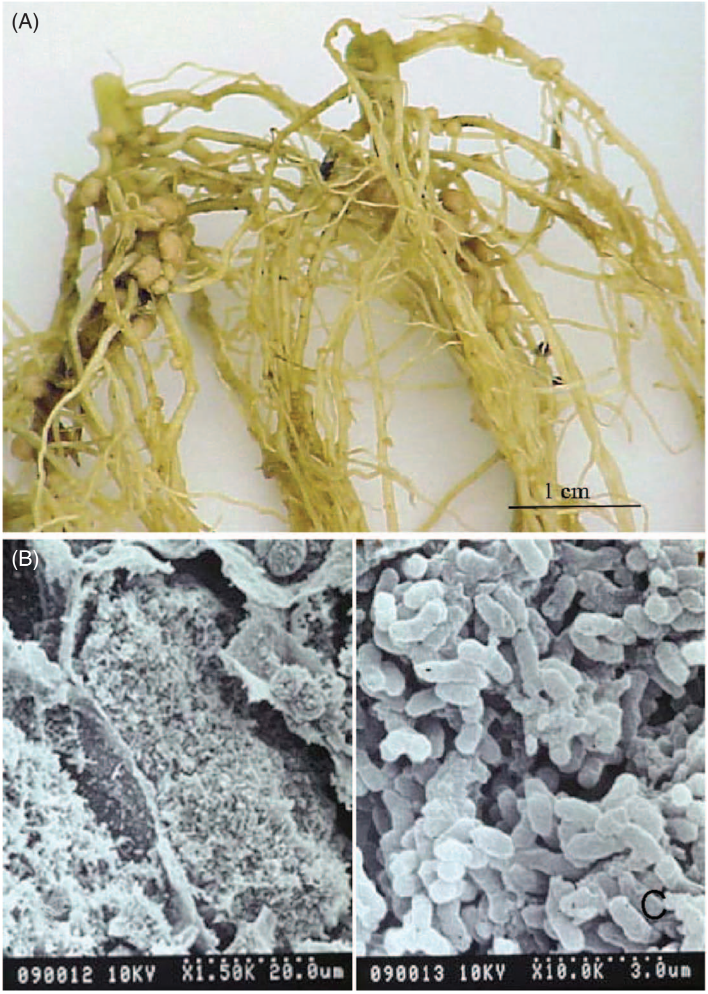



FIGURE 10.6 (A) Soybean root system with nodules of *Bradyrhizobium japonicum.* (Image courtesy of Pedro Antunes.) (B, C) Electron micrographs of alfalfa nodule interior, showing rhizobial bacteroids. (Images courtesy of Joe Muldoon.)

infections mainly result in determinate nodules. Determinate nodules are spherical in shape and ephemeral, lasting only a few days or weeks. Found, for instance, on soybean roots, these nodules contain vascular bundles that aid in the transport of C into the nodule and N out of the nodule. Indeterminate nodules are elongate and long-lived, possessing an apical meristem that continuously produces new cells. Indeterminate nodules have been studied on clover and have a more extensive vascular system than determinate nodules.

As with mycorrhizal symbioses, rhizobial associations are not always beneficial for the plant. Non-$N_2$-fixing strains of rhizobia are commonly found associated

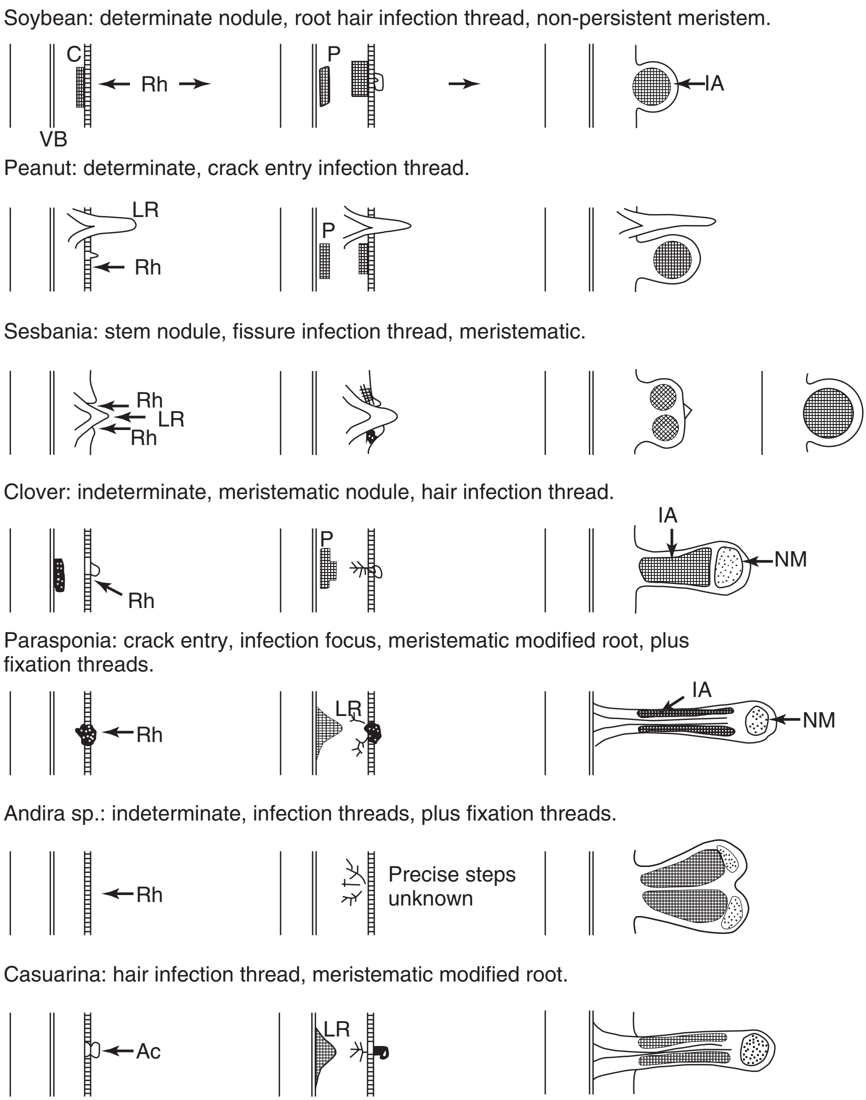



FIGURE 10.7 Schematic representations of different nodulation ontogenies. VB, vascular bundle; Rh, *Rhizobium* or *Bradyrhizobium* inoculant; Ac, actinomycete (i.e., *Frankia*) inoculant; C, cortex; P, pericycle-associated cell divisions; IA, infected area and region of nitrogen fixation; LR, lateral root (emerging or dormant) with associated root meristem; NM, nodule meristem. (Rolfe and Gresshoff, 1988. Reprinted with permission of Annual Reviews, www.annualreviews.org.)

with legumes and may be at a selective advantage over other strains infecting the same host because they avoid the energetically costly process of $N_2$ fixation. This is important from an agronomic standpoint: non-$N_2$-fixing strains may outcompete mutualistic strains for plant resources, resulting in reduced $N_2$ fixation at the whole

plant level. Compounding this issue, tillage can homogenize distributions of rhizobial communities, potentially increasing the frequency of crop plants infected by non-$N_2$-fixing strains (West *et al.*, 2002). Plant controls on this interaction are not absent, however; sanctions imposed by the host on these detrimental strains, as a result of reduced $O_2$ supply to non-$N_2$-fixing nodules, limit the fitness of cheating rhizobia and effectively stabilizes the mutualism (Kiers *et al.*, 2003).

## INTERACTIONS AMONG MUTUALISTS

None of the groups discussed above occur in isolation with the plant host; the activities of these organisms overlap spatially and temporally. Competition for infection sites, locations on the surface of roots where infection occurs and colonization begins, and for habitat within roots is a likely candidate for direct interactions among symbionts, while enhanced nutritional status of the host as a result of one symbiont may indirectly influence the formation and functioning of other symbioses.

Interactions among symbionts of plants during and following the colonization of root tissue influence the resulting composition of the endophytic microbial community. Plant populations and communities can support high levels of AM fungal species diversity, and multiple AM fungal species can be present on the same plant. However, the presence of more than one AM fungal species on individual root segments is thought to be uncommon (Wilson and Tommerup, 1992) and AM fungal species differ in their ability to colonize host roots in the presence of another AM fungal species (Lopez-Aguillon and Mosse, 1987). These observations suggest that, in addition to a variety of abiotic factors (P concentration, pH, water potential, and temperature), competition may be an important factor structuring AM fungal communities. The degree to which plant nutrition is improved varies among species and isolates of AM fungi (Wilson and Tommerup, 1992); therefore, the results of competitive interactions among AM fungi during the colonization of a host plant will have important consequences for the subsequent health of that individual plant, possibly with implications at community and ecosystem levels.

Klironomos (2003) demonstrated the varying responses of several plant species when individually colonized by different AM fungal species. The growth of individual plant species ranged from highly positive to highly negative relative to uninoculated control plants and was inconsistent across species of AM fungi (Fig. 10.5), suggesting a range of symbiotic outcomes from mutualistic to parasitic in arbuscular mycorrhizas. The mechanism behind this range of functional responses is not understood, but may be related to the degree of colonization and trade-offs associated with the extent of the hyphal network extending into the soil. AM fungal species vary in the biomass of extraradical hyphae produced; fungal associates that produce a larger hyphal network occupy a greater volume of soil and increase the potential for nutrient uptake but demand more photosynthetic C than associates

that produce a smaller extraradical hyphal network. Therefore, plant growth responses will depend on the overall benefits of expanding access to nutrients relative to the costs of maintaining the hyphal network. Differences in nutrient requirements and uptake ability among plant species likely contribute to the idiosyncratic nature of this interaction. As a result, stoichiometric responses in plant communities may be indirectly influenced by competitive interactions among AM fungal species during root colonization.

The spatial and temporal succession of EM fungi in a transplanted birch (*Betula* spp.) forest in Scotland has been well studied and suggests that interactions among EM fungi may influence the structure of EM communities (Deacon and Fleming, 1992). Diversity of fruit bodies increased progressively for up to at least 14 years. Fruiting bodies of EM species, observed near the base of the trees in previous years, appeared farther away from the tree base in successive years, being replaced in their previous distributions by fruiting bodies of previously unobserved species. *Lactarius pubescens* and *Hebeloma velutipes* were associated with younger roots and were thus found farther away from the tree base, while a *Cortinarius* sp. and *Russula* spp. were found associated with older roots near the tree base. A similar succession may occur within AM fungal communities. Johnson *et al.* (1991) observed that, along a field-to-forest chronosequence, *Acaulospora laevis*, *Acaulospora scrobiculata*, *Acaulospora spinosa*, *Glomus aggregatum*, *Scutellospora calospora*, *Scutellospora erythropa*, and *Scutellospora persica* spores were associated with early successional sites, while *Acaulospora elegans*, *Gigaspora gigantea*, *Glomus ambisporum*, *Glomus fasciculatum*, and *Glomus microcarpum* spores were associated with late successional sites; however, more study is required to determine if these apparent successional patterns are due to AM fungal interspecific interactions or just a shift of the AM fungal community in response to the changing plant community and/or abiotic characteristics of the chronosequence.

Competition among rhizobia has been studied with the goal of determining the necessary conditions for inoculant strains to nodulate hosts in the presence of indigenous strains, which may be inferior $N_2$ fixers. Several factors affect the outcome of competition for nodulation, including abundance; soil fertility, pH, temperature, and moisture; presence of predators, parasites, and antagonists; and pesticide use (Dowling and Broughton, 1986). The relative attractiveness of specific sugars, amino acids, and other components of the root exudates from host and nonhost plants differs among species of rhizobia. Strains also differ in motility, affecting the speed at which individual rhizobia encounter and nodulate the host. The presence of blocking strains, those that initiate infection thread formation and root hair curling but do not complete nodulation, can prevent competent rhizobia from nodulating the host plant.

Dual colonization of plants by AM fungi and rhizobia, in legumes, or *Frankia*, in nonlegumes, is common and has additive effects on nodulation, AM fungal colonization, and plant nutrition (Azcon-Aguilar and Barea, 1992; Dar *et al.*, 1997; Jha *et al.*, 1993; Siddiqui and Mahmood, 1995). The high P requirements in nodules during $N_2$ fixation (due to the upward of 25 to 30 mol of ATP required

per mole of $N_2$ fixed) and the N demanded by AM fungi during chitin synthesis suggest a physiological basis for this correlation between the presence of nodules and arbuscular mycorrhizas in root systems. Kucey and Paul (1982) observed that AM beans supported greater nodule biomass and greater rates of $N_2$ and $CO_2$ fixation than nonmycorrhizal beans. The greater rate of C utilization by nodules in AM beans (12% of total fixed C vs 6% in nonmycorrhizal beans) was offset by enhanced $CO_2$ fixation. Therefore, requirements for potentially limiting nutrients (C, N, P) in each individual symbiosis may be overcome by the presence of additional symbioses on the same host, enhancing the levels at which all processes occur and increasing the benefits to all participants in the tripartite symbiosis. The consequence of this tripartite symbiosis for the outcome of competitive interactions with plant species that participate in only one symbiosis is currently not known.

Molecular and biochemical investigations of tripartite symbioses have revealed a great deal of similarity between mycorrhizal and rhizobial symbioses, despite differences in morphological characteristics. Prior to colonization, signaling molecules derived from the AM fungal mycelium (putative "Myc factors") trigger similar responses in plants as to analogous molecules of rhizobial origin ("Nod factors"), as indicated by patterns of flavonoid accumulation (Vierheilig, 2004). In legumes, several of the same genes are induced during interactions with either rhizobia or AMF, and most nonnodulating mutants are also unable to form symbioses with AM fungi (Albrecht *et al.*, 1999). Recent research has uncovered divergence between AM fungi and rhizobia in terms of plant gene regulation, but also common patterns of gene induction between an AM fungus, *G. mosseae*, and an endophytic, plant-growth-promoting bacterium, *Pseudomonas fluorescens* (Sanchez *et al.*, 2004). The authors suggested that the observed patterns of divergence and similarity may be due to anatomical aspects of the symbioses, with nodule formation initiated by *Sinorhizobium melloti* requiring much more reorganization of root structures than during colonization by *G. mosseae* and *P. fluorescens*.

## INTERACTIONS WITH PATHOGENS

A disease is a deviation from the normal physiological status of an organism or its parts, resulting from the activity of biotic and abiotic factors, such that its vital functions are impaired. A variety of pathogenic soil microorganisms can cause diseases in plants, including viruses, bacteria, fungi, oomycetes, and nematodes. The interactions between several pathogens and plants are similar to the mutualisms discussed above in that they represent intimate relationships between organisms in which one colonizes the intra- or intercellular space within the roots, they can involve significant structural reorganization of root anatomy, and they require molecular signaling by both of the participants during the processes of recognition, infection, and colonization of roots. However, interactions resulting in disease are geared toward maximizing the fitness of one group while negatively affecting the host. Given the diversity of plant pathogens, no attempt will be made here to discuss

their individual life history characteristics, except in the context of their interactions with mutualistic plant associates and the resultant effects on plant population and community dynamics. For an introduction to these pathogens and their importance in plant pathology see Bruehl (1987).

Biological control of disease-causing organisms using plant mutualists has been, and continues to be, a goal of some researchers studying these mutualisms. As with most biological control agents, field trials frequently provide inconsistent and/or inconclusive results, although there have been several observations of reductions in disease-causing organisms and disease levels in crop plants following inoculation with plant mutualists. Laboratory studies suggest several mechanisms by which mutualists of plants reduce disease in plant hosts: directly, via antagonism or antibiosis, or indirectly, via enhanced nutritional status of the host, competition for infection sites or limiting nutrients in the rhizosphere, alteration of root morphology, and induction of host defenses. Most studies investigating interactions between disease-causing organisms and plant associates and their consequences for host health have focused on plant-pathogenic (PP) fungi and nematodes. Few studies have focused on interactions with bacterial and viral pathogens or root herbivores. Some AM fungi have been observed to reduce infection of soybean, eggplant, cucumber, mulberry, and grape by *Pseudomonas* spp. AM hosts tend to exhibit elevated viral loads and greater levels of viral diseases, hypothesized to be due to higher P levels in AM plant tissue improving host quality and facilitating viral growth (Xavier and Boyetchko, 2002). In addition, a few studies have shown reduced survivorship of larval insects feeding on AM roots relative to those feeding on nonmycorrhizal roots (see Gange and Bower, 1996, for a brief review).

There are several papers that chronicle the effects of EM and AM fungi on populations of PP fungi and disease severity. Dehne (1982) summarized the literature pertaining to the impact of mycorrhizal fungi on PP fungi and found that there was evidence for both increased and decreased levels of PP fungi and plant disease in the presence of mycorrhizal fungi. Increased disease levels were suggested to be due to enhanced nutritional status of the host, making it a higher quality resource for the pathogenic fungus. Reduced disease levels might be explained by enhanced host vigor, interference with pathogen infection, or competition for habitat within roots. For EM fungi, there is also the possibility of direct chemical antagonism of pathogenic fungi (Duchesne *et al.*, 1988).

Interactions between AM fungi and PP nematodes appear to be symmetrical, with the outcome dependent on the order of infection. The two groups appear to be incompatible, competing for habitat within the roots or indirectly altering the quality and/or quantity of habitat available for the other group. AM fungi are unable to colonize root segments parasitized by endoparasitic nematodes. AM structures are usually not found in galled tissues inhabited by galling nematodes, whether live, inactive, or dead, suggesting that physiological changes involved in the formation of root galls make these roots inhospitable to AM fungi. Ectoparasitic nematodes inflict damage to root tissue such that AM fungal colonization of these damaged roots is reduced.

PP nematodes have, on occasion, been observed to infect AM roots. The colonization of roots by AM fungi can lead to reduced populations of PP nematodes and lowered disease severity, although this interaction is often cultivar-specific and dependent on soil nutrient levels. Nematode life history is also important in this interaction, with AM fungi reducing population growth of sedentary endoparasitic nematodes but not migratory endoparasitic nematodes in a meta-analysis of 17 independent studies (Borowicz, 2001). AM fungi may increase plant tolerance of PP nematodes; nematode infection results in local alterations to root architecture that interfere with nutrient uptake, which may be mediated at the whole plant level by AM fungal colonization of other parts of the root system. However, at least some benefits of AM fungal colonization are likely associated with mechanisms other than increased plant vigor since benefits to plants in the presence of AM fungi are not negated by increasing nutrient availability (Ingham, 1988). In a few cases, increased populations of PP nematodes were observed to be associated with AM fungal colonization; this positive response may be a result of AM fungi increasing root mass and, thus, habitat availability for PP nematodes. Therefore, to properly interpret the impact of AM fungal colonization on root pathogens, estimates of PP nematode abundance need to be standardized with estimates of root mass.

Newsham *et al.* (1995) proposed a dichotomy in the type of benefit derived by plants from AM symbioses based on root system architecture (Fig. 10.8). Highly branched root systems, which are highly susceptible to pathogen infection, benefit from AM fungi occupying locations in the roots where pathogens could otherwise

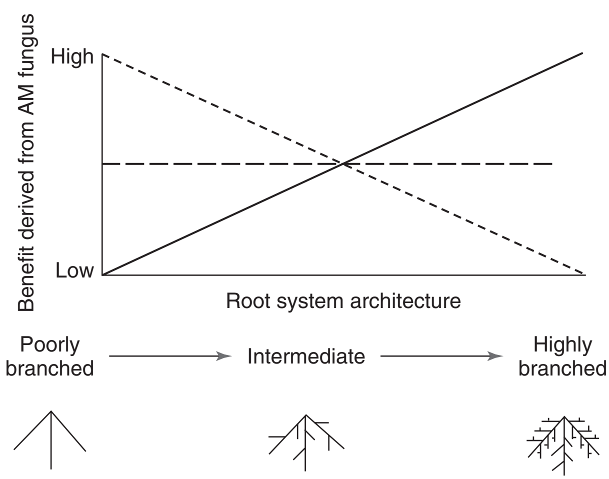



FIGURE 10.8 Hypothetical P uptake (dashed diagonal line) and pathogen protection (solid diagonal line) benefits of AM fungi for plant species with poorly branched to highly branched root system architectures. The dashed horizontal line represents other functions of the AM association not defined by root system architecture. (Reprinted from Newsham, Fitter, and Watkinson, 1995, copyright 1995, with permission from Elsevier.)

colonize. Poorly branched root systems, which are less susceptible to pathogen infection, but occupy a relatively low volume of soil, benefit primarily as a result of P uptake by AM fungi. The authors use three pieces of evidence to support their model: (1) a positive correlation between P inflow and specific root tip number (a measure of root system branching) for seven forb and grass species; (2) a positive correlation between P uptake and percentage root length colonized by AM fungi in two plant species with poorly branched root systems, *Hyacinthoides non-scripta* and *Ranunculus adoneus*; and (3) the observation that a *Glomus* sp. provides pathogen protection to *Vulpia ciliata,* an annual grass with a highly branched root system, yet does not enhance P uptake. However, further tests of this model have not been conducted.

Although less studied than mycorrhizal fungi for their potential to reduce pathogen loads on host plants, rhizobia can reduce infection rates by, and enhance host tolerance to, pathogenic fungi. This enhanced tolerance is probably due to greater plant health and the stimulation of defense compounds, but possibly also to production of antifungal compounds. For instance, rhizobitoxine plays a role in nodule development, but has also been observed to inhibit growth of *Macrophomina phaseolina* in culture plates (Chakraborty and Purkayastha, 1984). Field trials using selected species of *Rhizobium* and *Bradyrhizobium* resulted in reduced infection rates of host and nonhost crops (including nonleguminous crops) by *M. phaseolina*, *Rhizoctonia solani*, and *Fusarium* spp. (Ehteshamul-Haque and Ghaffar, 1993). Fungal growth was reduced in culture, suggesting that competitive or antagonistic interactions between rhizobia and fungi may have played an important role in reducing infection rates. Rhizobia have been observed to be stimulated by and migrate toward roots of nonlegumes, perhaps explaining the reduced infection rates of nonleguminous crops.

## IMPLICATIONS FOR PLANT POPULATIONS AND COMMUNITIES

Much of the study of plant–microbial mutualisms has focused on interactions at the level of individual plants, yet the effects of these mutualisms are also observed in plant populations and communities. Plant population dynamics are driven by a number of factors (e.g., competition with other plants, herbivory, resource availability) that are mediated or strengthened by mycorrhizal fungi and rhizobia. Plants that engage in symbioses with N-fixing bacteria are able to thrive under conditions of low N availability but also foster conditions, through the process of $N_2$ fixation, that eventually allow for the growth of other plant species and facilitate succession. Complex interactions among plants and AM fungi influence the structure of plant communities; increasing AM fungal species diversity enhanced plant species diversity in abandoned agricultural fields, likely due to interactions between individual plant and fungal species modifying competitive interactions among plants (van der Heijden *et al.*, 1998).

Recent research has been focused on elucidating the roles of plant–microbial mutualisms in invasion by nonnative species. Nonnative plant species can use mycorrhizal fungi to facilitate invasion of plant communities. For example, AM fungi improve the competitive ability of invasive spotted knapweed (*Centaurea maculosa*) against some native grass and forb species (Callaway *et al.*, 2004). Other nonnative plants disrupt mycorrhizal mutualisms to prevent recruitment of native plants. For example, garlic mustard (*Alliaria petiolata*) produces allelochemicals that have negative effects on AM fungal communities, reducing growth of native seedlings (Stinson *et al.*, 2006). In some cases, the establishment of nonnative plants is limited by the absence of mutualists in the nonnative range. Establishment in New Zealand and South Africa of nonnative *Pinus* spp. is limited to areas where suitable EM fungi have either invaded or been introduced, and invasiveness of actinorrhizal plants is linked to their ability to form symbioses with native *Frankia* spp. (Richardson *et al.*, 2000).

In some cases, our basic understanding of plant-microbial mutualisms has already been applied for practical purposes. There has been some success in the use of these organisms, either inundatively in small-scale plantings or as inoculants prior to the distribution of seedlings or rootstock to growers. Strains of *Bradyrhizobium japonicum* are commercially available as an inoculant to promote nodulation of legume field and greenhouse crops. Inoculation of plant seedlings with mycorrhizal fungi prior to transplantation can be a useful technique to enhance productivity of orchard crops and to promote reforestation and revegetation efforts, such as those for mine spoils. Also, with a greater understanding of how symbionts of plants respond to disturbances of anthropogenic origin (e.g., climate change, N deposition, introduction of invasive plant species), it may be possible to predict how the structure and functioning of natural and agricultural ecosystems will be influenced by these disturbances and to take the appropriate steps to prevent or mediate their effects.

## CHALLENGES IN THE STUDY OF INTERACTIONS

A major problem associated with studying interactions involving plant–microbial mutualisms is our inability to monitor accurately and precisely the population dynamics of soil and root-associated microorganisms (see Chaps. 3 and 4). Indirect measures of interaction strength focus on functional responses, such as disease severity or growth response of the plant following inoculation with different combinations of microbes. Direct measures, such as counting bacteria or measuring fungal biomass, are problematic because they tend to overestimate viable population size. Culture-dependent methods work only for the small fraction of soil microorganisms that can be cultured on artificial media. AM fungi are obligate symbionts and can be cultured only on living roots, so abundance is estimated by the extent to which fungi colonize roots, which provides no means of determining the actual

TABLE 10.2 Comparison of Culture-Based and Molecular Techniques for Estimating the Effect of *Glomus intraradices* on the Abundance of *Fusarium solani* f. sp. *phaseoli*

| Soil compartment | Treatment | *F. solani* abundance (CFU × 1000/g soil) | *F. solani* abundance (μg DNA/g soil) |
|---|---|---|---|
| Rhizosphere | *Glomus* present | 2.52[a] | 7.89[a] |
| | *Glomus* absent | 1.95[a] | 33.03[b] |
| Mycosphere | *Glomus* present | 3.12[a] | 12.40[a] |
| | *Glomus* absent | 4.39[a] | 93.69[b] |
| Bulk soil | *Glomus* present | 2.79[a] | 17.65[a] |
| | *Glomus* absent | 2.34[a] | 46.83[b] |

Number of colony-forming units (CFU) was estimated on Nash–Snyder medium supplemented with benomyl, while quantitative PCR was used to quantify *F. solani* DNA. Different letters indicate that *F. solani* abundance, in the sampled soil compartment, differed significantly between the two *Glomus* treatments (data from Filion *et al.*, 2003).

abundance of AM fungi in soil, or by spore counts, which may overestimate the abundance of viable propagules.

Technological advances are aiding our understanding of plant–microbial mutualisms. Physiological and molecular markers (e.g., phospholipid fatty acids, proteins) allow for some resolution of microbial identity and abundance in soil and roots. Recent advances in the isolation and quantification of DNA from soil and plant tissue will be extremely valuable for studying the population and community dynamics of soil and root-associated microorganisms in real time. For example, Filion *et al.* (2003) developed a protocol using quantitative polymerase chain reaction (PCR) to study interactions between *G. intraradices* and *F. solani.* Using this protocol, plants inoculated with *G. intraradices* supported measurably reduced population levels of *F. solani*, corresponding to reductions in disease severity, that were not detected using culture-dependent techniques (Table 10.2). Soil community fingerprinting using PCR-based techniques (e.g., degrading-gradient gel electrophoresis, terminal restriction fragment length polymorphism) and comparing cloned gene sequences to existing sequence databases are other ways of characterizing microbial communities and determining how components of the soil community differ in response to different biotic and abiotic factors (Fig. 10.9).

## CONCLUSIONS

A diverse assemblage of plant–microbial mutualisms exists in the rhizosphere and rhizoplane. A primary focus of ecological research into plant–microbial mutualisms has been to understand the role that mutualistic symbioses play in promoting plant productivity. Recent research, however, has illuminated the fact that these symbioses likely function along a continuum, from mutualism to parasitism; this new view has enhanced our understanding of how plant communities are structured

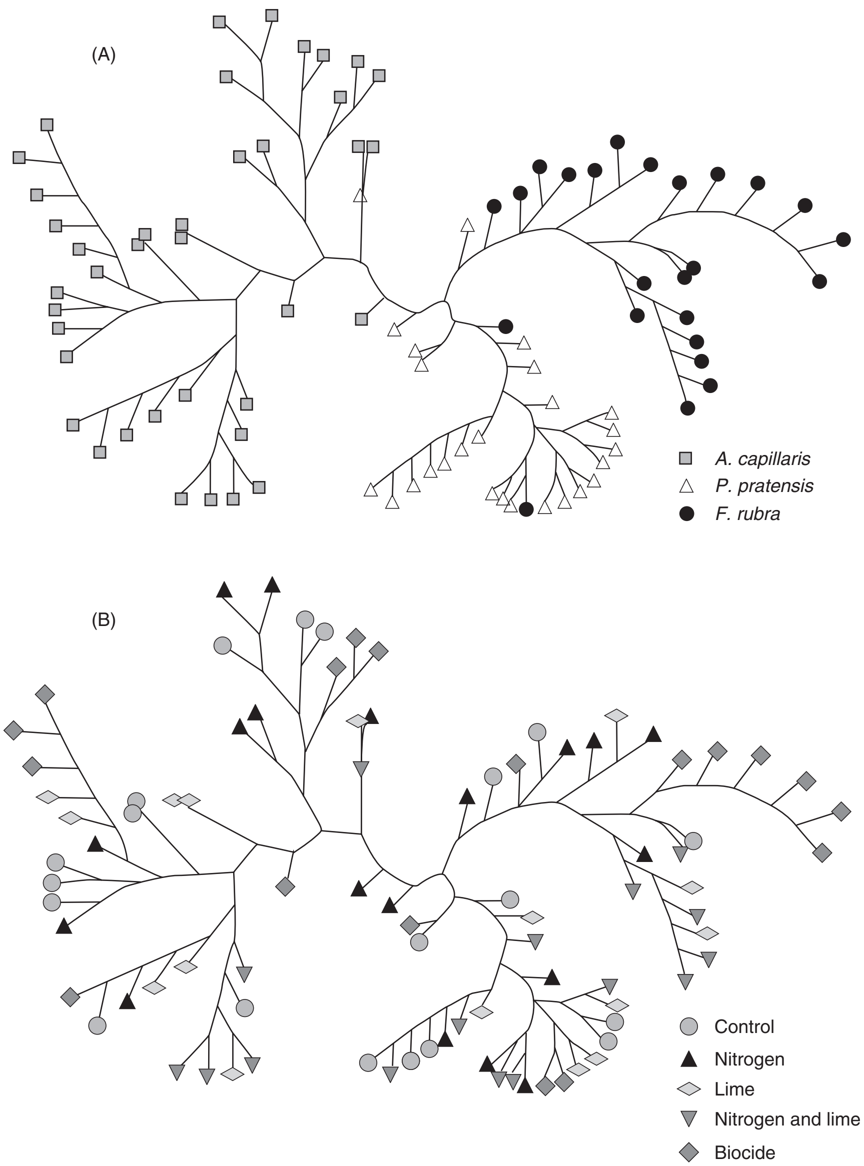



FIGURE 10.9 Influence of (A) plant species and (B) soil treatment on the structuring of AM fungal communities, based on the similarity (maximum parsimony) of terminal restriction fragment length polymorphism profiles from individual roots of *Agrostis capillaris*, *Poa pratensis*, and *Festuca rubra.* Each terminal corresponds to the AM fungal community in a single root. Roots belonging to the same plant species were inhabited by similar AM fungal communities relative to those of other plant species. Soil treatment did not appear to have as great an effect on the AM fungal community as did host species. (Reprinted from Vandenkoornhuyse *et al.*, 2003, with permission; copyright 2003, Blackwell Publishing.)

and may affect how mutualistic symbioses are exploited in agriculture and forestry. The ubiquity of mutualistic symbioses in the rhizosphere and rhizoplane, as well as the high levels of microbial diversity and activity in general, guarantees that these regions are rich in interactions, many of which influence plant growth and fitness, community dynamics, and ecosystem functioning. A greater understanding of these interactions could allow us to better predict the effects of anthropogenic disturbances on the functioning of ecosystems and may lead to more effective microbial inoculants for use in agriculture and restoration. However, our ability to fully understand these interactions has been hampered by the opaque nature of the soil environment, the heterogeneity of soil, and the sheer number of linkages in these interactions. Also confounding matters is our inability to culture the majority of organisms present within the soil. New technologies show great promise for characterizing the composition of microbial communities associated with roots and soil and should allow us to resolve some of the interactions among mutualists, other soil organisms, and plants that are occurring in the soil environment.

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# 11

# Spatial Distribution of Soil Organisms

Serita D. Frey



## INTRODUCTION

Soil biota, which represent a large proportion of Earth's biodiversity, have a worldwide distribution. Once thought to be restricted to the top few meters of terrestrial ecosystems, soil organisms are now known to survive and grow in some seemingly unlikely and often inhospitable places, including in the canopies of tropical forest trees, in deep subsurface environments, in recently deposited volcanic materials, under deep snow in alpine systems, in Antarctic Dry Valley soils, and in cryoconite holes, pockets of meltwater containing windblown soil on the surface of glaciers (Fig. 11.1). While there are still large gaps in our understanding of how soil organisms are distributed, there has been a dramatic increase in information obtained in this area over the past decade. This chapter summarizes what is known about the distribution of soil biota, from geographic differences at the regional and global scale down to variability in microbial populations at the microscale.

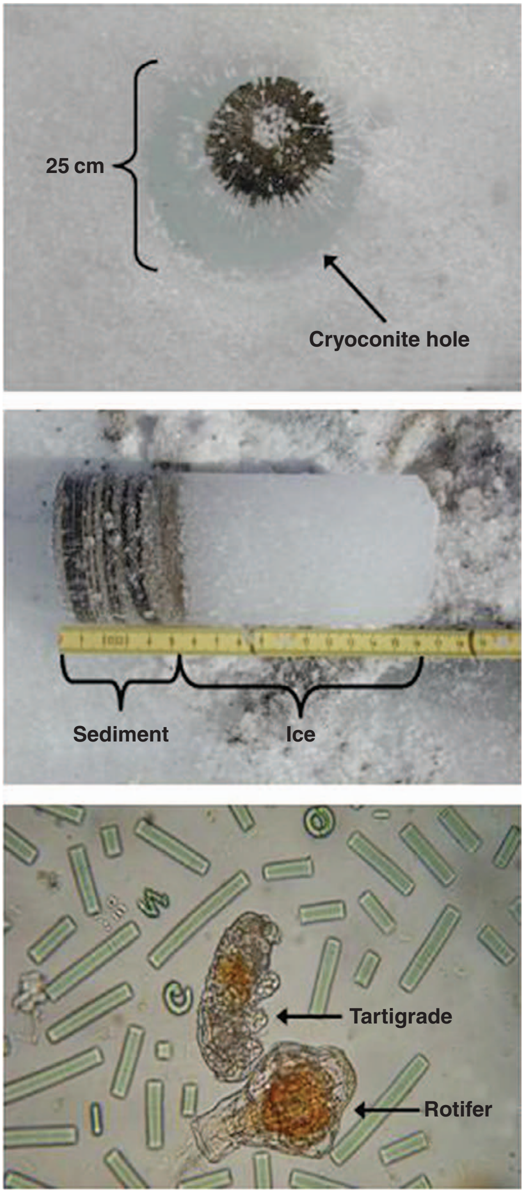



FIGURE 11.1 Cryoconite hole formed on the surface of an Antarctic glacier. (Top) The ice of the cryoconite hole reflects light differently compared to the surrounding ice, making it easy to spot on the glacial surface. (Middle) Ice core from the cryoconite hole showing the accumulation of windblown sediment at the top of the hole. (Bottom) Organisms associated with sediments in the cryoconite hole. The green rods are cyanobacteria. (Photos courtesy of Dorota Porazinska, University of Florida, and Thomas Nylen, Portland State University. Used by permission.)

## GEOGRAPHICAL DIFFERENCES IN SOIL BIOTA

Most macroscopic plant and animal species have a restricted geographic distribution because of natural barriers to migration (e.g., mountain ranges) and climate sensitivity. This isolation has, over geological time, led to the evolution of new species and the development of geographically distinct plant and animal communities. Global distributions of most of the world's flora and fauna are generally known. There has been much less emphasis on understanding and mapping the biogeography of microscopic organisms. Although molecular analysis has revealed that microbial diversity in soils far exceeds that of macroscopic organisms, the geographical patterns of this diversity and the factors controlling these patterns are only beginning to be examined. Most studies that have been done have focused on human and animal pathogens (e.g., *Escherichia coli*, *Haemophilus influenzae*). A common perception is that microorganisms are cosmopolitan in their distribution, being capable of growth in many different places worldwide. This idea goes back more than a century to Martinus Beijerinck, a Dutch soil microbiologist, who suggested that "everything is everywhere, the environment selects," meaning that microbial species can be found wherever their environmental requirements are met. However, the concept of a common occurrence of soil microorganisms may be more apparent than real due to a lack of information on microbial distributions. As more details of microbial populations are delineated with biochemical and molecular techniques, it may be found that at least some soil organisms are restricted to specific geographical areas (i.e., they are endemic).

Certainly microorganisms have had the opportunity over time to become distributed worldwide. Due to their small size and large numbers, they are continuously being moved around, often across continental-scale distances. Dispersal mechanisms include water transport via rivers, groundwater, and ocean currents; airborne transport in association with dust particles and aerosols, especially during extreme weather events such as hurricanes and dust storms; transport on or in the intestinal tract of migratory birds, insects, and aquatic organisms; and human transport through air travel and shipping. Even isolated environments, like those found in the Antarctic, show a wide range of microbial species that appear to have been introduced from other places. Of 22 fungal genera identified in Antarctic soil samples, 12 taxa were found exclusively in the area surrounding the Australian Caseyu Research Station (Azmi and Seppelt, 1998). These fungi, dominated by *Penicillium* species, were presumably introduced into this environment by human visitors to the station. Thus almost any microbial species has the means to achieve widespread dispersal.

Further support for the idea that microorganisms have global distributions comes from a study of protozoa living in the sediments of a crater lake in Australia (Finlay *et al*., 1999). This system is geographically isolated from northern Europe, where most known protozoan taxa have been isolated and identified. Of 85 species collected from the Australian system, all had been previously described and are

known from northern Europe. They apparently reached the isolated crater by dispersal from other freshwater, soil, and marine environments. Another argument for the cosmopolitan distribution of protozoa is the relatively low number of species found globally. For example, there are about 3000 known species of free-living soil ciliates. This is in comparison to 5 million insect species, many of which have geographically restricted ranges. The implication here is that lower endemicity in protozoa results in low global species richness since geographic isolation leading to speciation will be rare (Finlay, 2002).

While it is generally agreed that many protozoan species are cosmopolitan in their distribution, this may not be the case for other microbial groups. Heterotrophic soil bacteria, for example, have been shown to exhibit strict site endemism. Fluorescent *Pseudomonas* strains isolated from soil samples collected at 10 sites on four continents showed no overlap between sites. The same genotype was found only in other soil samples from the same site and not at other sites in a region or on other continents (Cho and Tiedje, 2000). Intraspecific differences in the optimum growth temperature, pH, and substrate ($NH_4^+$) concentration for the nitrifiers are also known to exist. *Penicillium* is abundant in temperate and cold climates. *Aspergillus* predominates in warm areas. *Fusarium* wilt of bananas is inhibited in areas where the clay mineral smectite predominates. Cyanobacteria are commonly found in neutral to alkaline soils, but rarely under acidic conditions. The fungal component of the microbial community appears to be particularly susceptible to changes in soil conditions. For example, fungal biomass and fungal-derived organic matter have been shown to be positively related to soil moisture as influenced by gradients in mean annual temperature and precipitation (Figs. 11.2 and 11.3). Thus regional differences do reflect the ability of soil organisms to respond to specific environmental controls.

In addition to species distributions, it is useful to consider regional and global patterns in soil microbial biomass, which represents between 2 and 5% of total terrestrial soil C. Climate, vegetation, soil characteristics, and land-use patterns all interact to influence microbial abundance and biomass at a given location. Microbial biomass is generally positively related to soil organic matter contents in most ecosystems, peat and organic soils being an exception. Levels of microbial biomass are also typically correlated with soil clay content. Clay minerals promote microbial growth by maintaining the pH in an optimal range, buffering the nutrient supply, adsorbing metabolites that are inhibitory to microbial growth, and providing protection from desiccation and grazing through increased aggregation. Total amounts of microbial biomass are also impacted by land use, with lower levels typically observed in arable compared to undisturbed forest and grassland soils due to cultivation-induced losses of organic matter. Microbial biomass is also correlated with latitude, with microbial biomass tending to be lower but more highly variable at high latitudes. This increased variability in microbial biomass with increasing latitude is attributed to higher interseasonal variation in temperature (Wardle, 1998).

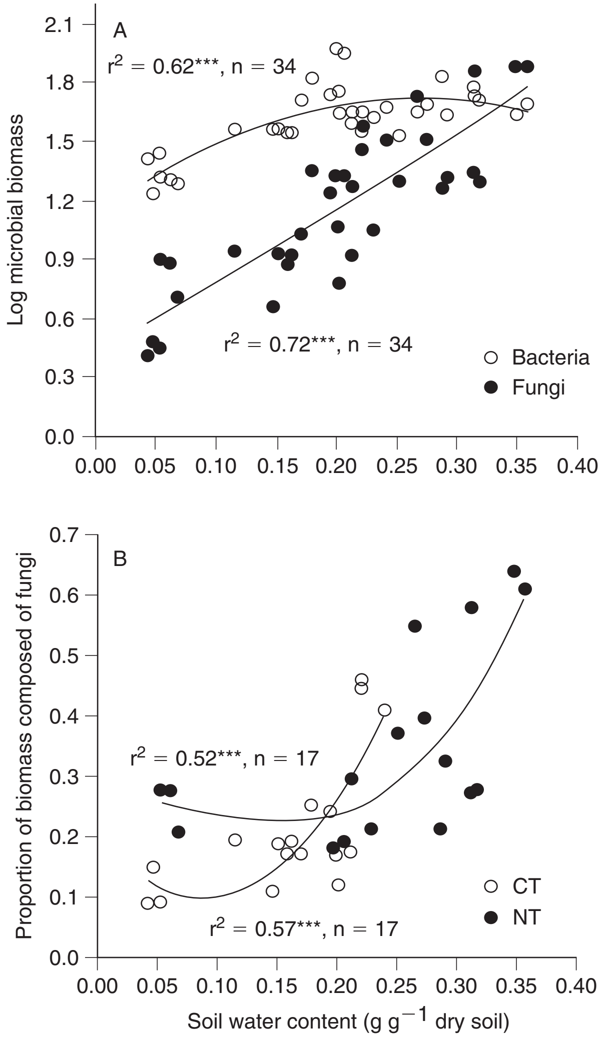



FIGURE 11.2 Relationship between soil water content at the depth of 0–5 cm and (A) bacterial and fungal biomass C and (B) the proportion of total microbial biomass composed of fungi for soils collected from six long-term tillage comparison experiments in North America. CT, conventional tillage; NT, no tillage. (From Frey *et al.*, 1999. Used by permission.)

## ASSOCIATION OF SOIL ORGANISMS WITH PLANTS

The sum total of phenomena occurring on or near plant roots has a great impact on both plant growth and microbial abundance, activity, and community composition

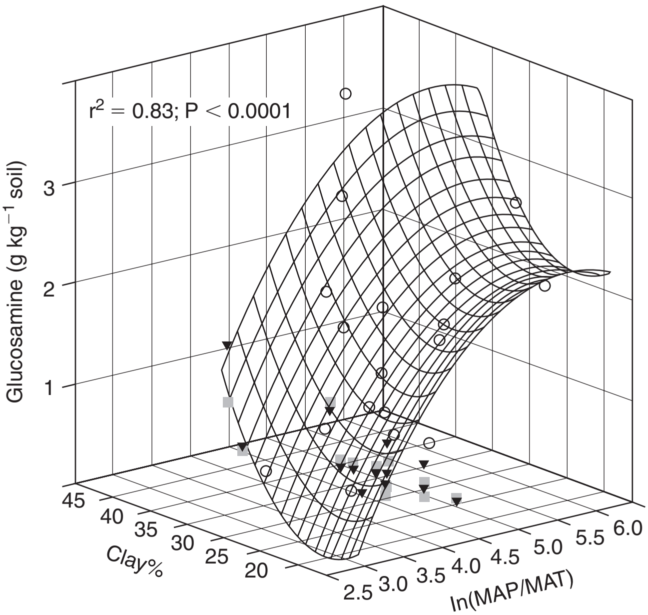



FIGURE 11.3 Glucosamine concentrations (g kg$^{-1}$ soil) in soil of grassland ecosystems (○), no-tillage agroecosystems (▼), and conventional tillage agroecosystems (■) and their relationship with clay content, mean annual precipitation (MAP), and mean annual temperature (MAT). The grassland data were obtained from Amelung *et al.* (1999). (Figure from Six *et al.*, 2006. Used by permission.)

(Fig. 11.4). Hiltner, in 1904, used the term rhizosphere for the area of bacterial growth around legume roots. Later the rhizosphere became generally known as the soil region under the immediate influence of plant roots and in which there is a proliferation of microorganisms due to the influx of plant-derived labile organic matter into the soil. Pathways for release of plant assimilates from roots include turnover of fine roots, leakage or diffusion of molecules across cell membranes (exudation), and sloughing off of cells and tissue fragments during root growth. Root caps and tips are sites of active exudation, releasing mucilaginous material as well as root caps and cells. Analyses of the organic materials found in the rhizosphere reveal a wide assortment of compounds, including aliphatic, amino, and aromatic acids and amides, sugars, amino sugars, cellulose, lignin, and protein.

Complex interactions and feedbacks occur at the plant–soil interface, representing potentially important factors regulating ecosystem structure and functioning. These interactions range from mutualistic to pathogenic and have long been recognized for their role in plant nutrition and nutrient cycling. More recently, plant–microbe interactions have been found to influence the composition and diversity of both plant and soil microbial communities. Microbial abundance, activity, and community composition and diversity often reflect the plant species present in a given soil. For example, bacteria isolated from the rhizospheres of different grass species

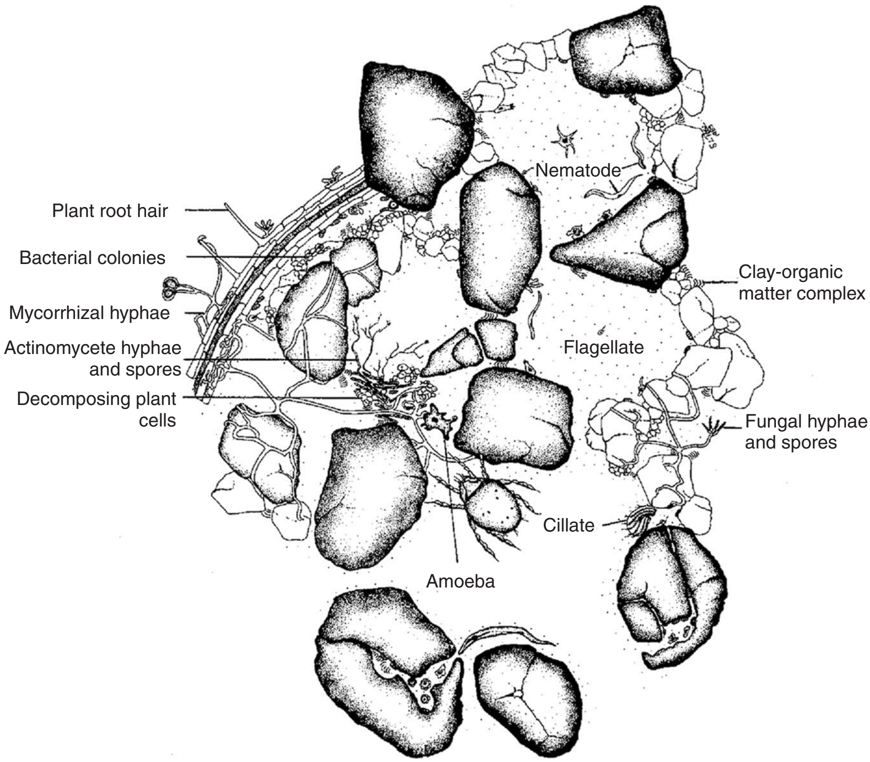



FIGURE 11.4 Diagrammatic representation of a plant root and associated biota in an approximately 1-$cm^2$ area. (Adapted from S. Rose and T. Elliott, personal communication.)

exhibited differential growth and C source utilization patterns (Westover *et al.*, 1997). Plant-mediated differences in the microbial community are potentially attributable to specific variations between plant species in the quality and quantity of organic matter inputs to the soil. Plant resource quantity and quality can also be altered by disturbances such as herbivore grazing and global change (e.g., elevated atmospheric $CO_2$, N deposition). Plant herbivory has been shown to increase C allocation to roots, root exudation, fine root turnover, soil-dissolved organic C, microbial biomass and activity, and faunal activity (Bardgett and Wardle, 2003). These changes in turn alter soil N availability, plant N acquisition, photosynthetic rates, and, ultimately, plant productivity.

Just as the plant community may be a determinant of microbial community structure, the diversity and composition of the microbial community may play a role in plant community dynamics. The diversity of arbuscular mycorrhizal fungi was observed to be a major factor contributing to plant diversity, above- and below-ground plant biomass, and soil nutrient availability in a macrocosm experiment simulating North American old-field ecosystems (Van der Heijden *et al.*, 1998).

An increase in arbuscular mycorrhizal fungal diversity was accompanied by a significant increase in the length of mycorrhizal hyphae in the soil leading to greater soil resource acquisition. As the number of mycorrhizal fungal species increased, plant diversity, biomass, and plant tissue phosphorus content increased, while the phosphorus concentration in the soil decreased.

There is accumulating evidence that plants "culture" a soil community that then controls their long-term survival and growth, sometimes negatively. Survival and growth of several grass species were significantly reduced when grown with their own soil community rather than that of another plant species (Bever, 1994). This result was attributed to an accumulation of specific plant pathogens or a change in microbial community composition. Such a negative feedback may provide a mechanism for maintenance of plant communities in natural ecosystems, whereby an individual plant cannot dominate a community because of the accumulated detrimental effect of the soil community on plant growth. It has been hypothesized that exotic plants that become invasive have escaped control by local soil organisms at invaded sites and may even alter the microbial community to their benefit. Callaway *et al.* (2004) reported that spotted knapweed (*Centaurea maculosa*), an invasive weed in North America, cultivates a soil community in its native European soil that negatively affects its own growth, possibly controlling its spread in its home range. However, the plant cultivates a different soil community at invaded sites in the western United States, positively enhancing its own growth and contributing to its success as an invasive species.

## SPATIAL HETEROGENEITY OF SOIL ORGANISMS

Organism abundance and activity are not randomly distributed in soil, but vary both horizontally and vertically through the soil profile (Fig. 11.5). Different groups of organisms exhibit different spatial patterns, because they each react to soil conditions in different ways (Klironomos *et al.*, 1999). This spatial heterogeneity, which has been observed at the scale of millimeters to hundreds of meters, has been shown in some studies to correlate with gradients in site and soil properties, including bulk density, aggregation, texture, oxygen concentration, pH, moisture, soil organic matter content, inorganic N availability, precipitation levels, and vegetation dynamics. Some of these properties are important at the microscopic scale, whereas others act over larger distances. For example, microbial biomass and collembolan abundance in an agroecosystem reflected large-scale gradients in soil C content and cultivation practices (Fromm *et al.*, 1993). In other cases, soil characteristics have been found to explain a relatively minor amount ($<30\%$) of the spatial variation in organism abundance (Robertson and Freckman, 1995). Spatial heterogeneity can be high even in soils that appear relatively homogeneous at the plot or field scale (Franklin and Mills, 2003). Nematode populations were strongly

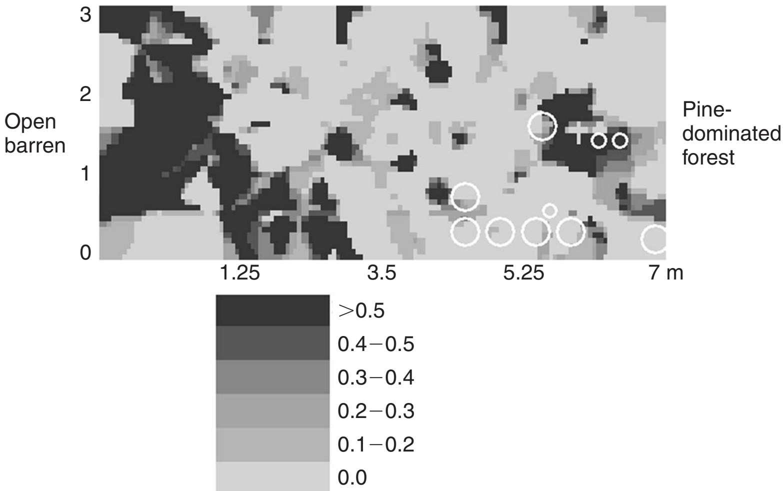



FIGURE 11.5 Spatial distribution of ectomycorrhizal fungi in a 7 × 3-m transect from the edge of a pine-dominated forest across an open serpentine barren at Soldiers Delight Natural Environment Area, a serpentine complex in Owings Mills, Maryland, USA. Units are percentage colonization of pine seedling root tips. Circles represent pine trees. (Courtesy of Rachel Thiet, Antioch New England Graduate School, Keene, NH, USA. Used by permission.)

patterned in an agroecosystem despite many years of soil tillage and monoculture cropping, suggesting that spatial heterogeneity may be even higher in less disturbed systems (Robertson and Freckman, 1995).

There is little detailed information available on the spatial distribution of soil biota, and spatial variability has historically been regarded as random noise in the system. Geostatistics provide a method for quantifying spatial heterogeneity and providing information on the underlying causes of observed spatial patterns. Information on spatial patterns can be used to design more statistically powerful experiments, to improve our understanding of how soil communities develop, and to determine what factors are important for regulating and maintaining soil function. It has been hypothesized that high levels of soil biodiversity are attributable to spatial heterogeneity in resource availability as influenced by land-use patterns and plant community dynamics (Ettema and Wardle, 2002). In particular, spatial isolation may be an important determinant of microbial community structure by facilitating species coexistence. In a simulated soil environment, one bacterial species dominated the community under saturated conditions where the pore network was highly connected (Treves *et al.*, 2003). However, under low moisture conditions (i.e., discontinuous water films) spatial isolation of microbial populations allowed a less competitive species to become established in the community. These results concur with the observation that saturated subsurface soils have lower microbial diversity compared to unsaturated surface soils (Zhou *et al.*, 2004).

## VERTICAL DISTRIBUTION WITHIN THE SOIL PROFILE

The abundance and biomass of most soil organisms are highest in the top 0–10 cm of soil and decline with depth in parallel with organic matter contents and prey availability. Approximately 65% of total microbial biomass is found in the top 25 cm of the soil profile. Below that depth, microbial densities typically decline by 1–3 orders of magnitude (Fig. 11.6). Hyphal density of and root colonization by mycorrhizal fungi decrease substantially below 20 cm. Mycorrhizal fungal spores are typically not found below the plant rooting zone. Numbers of microbial grazers (e.g., protozoa, collembola) also decrease with depth, often more rapidly than either their bacterial or their fungal prey. For example, collembolan numbers peak at 1–5 cm below the soil surface and drop to almost none below 10 cm. While generally low, the numbers and activities of soil organisms at depth vary spatially depending on gradients in texture, pH, temperature, water availability, and organic matter content. Interfaces between layers often generate localized regions of greater saturation, where microorganisms may exhibit increased numbers or activity due to improved access to nutrients.

In addition to abundance and activity, microbial community composition and diversity also change across the soil profile. Abundances of gram-negative bacteria, fungi, and protozoa are highest at the soil surface, while gram-positive bacteria and actinomycetes tend to increase in proportional abundance with increasing depth (Fierer *et al.*, 2003). Microbes in deeper soil horizons tend to be more C limited than surface microorganisms. Mycorrhizal species change along a vertical gradient, differing in their preference for the organic or mineral soil layers. Only 4 of 22

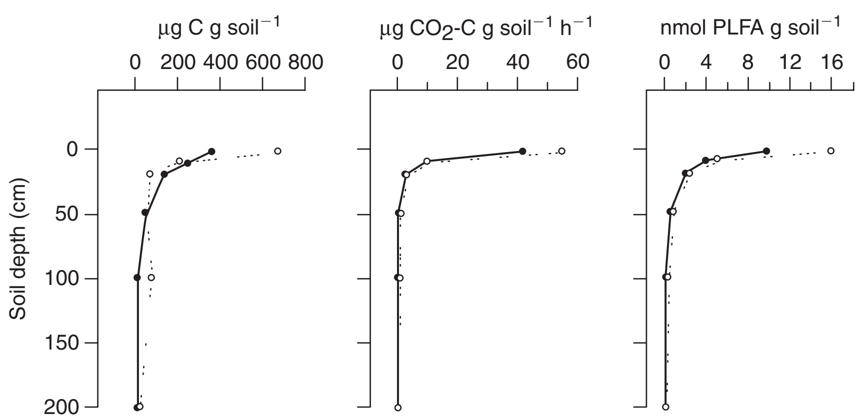



FIGURE 11.6 Microbial biomass with depth for a valley (open circles) and terrace (closed circles) soil profile as determined by three methods. (Left) Chloroform fumigation extraction. (Middle) Substrate-induced respiration. (Right) Phospholipid fatty acid (PLFA) analysis. (Adapted from Fierer *et al.*, 2003.)

ectomycorrhizal fungal taxa identified in a coniferous forest soil profile were found on root tips collected to a depth of 53 cm (Rosling *et al.*, 2003). Two taxa were restricted to the organic horizon, while 11 were found only in the mineral soil. Soil invertebrate communities also vary with depth, particularly in the organic and upper mineral soil horizons. For example, there is a succession from litter-dwelling to soil-dwelling species of collembola at the interface between organic and mineral soil layers. Gut content analysis has shown that collembola near the top of the O horizon feed preferentially on pollen grains, while those at the bottom of this layer feed mainly on fungal material and highly decomposed organic matter (Ponge, 2000).

In arid and semiarid regions, soil biota are widely distributed as biological soil crusts (also called cryptogamic, microbiotic, microphytic crusts), which form a protective soil covering (see Fig. 11.7). Soil crusts consist of a specialized community of cyanobacteria, green and brown algae, mosses, and lichens. Sticky substances produced by crust organisms bind surface soil particles together forming a continuous layer that can reach 10 cm in thickness. Cyanobacteria in arid surface soil from the badlands in Spain were recently shown to migrate vertically to and from the soil surface in response to changing moisture conditions (Garcia-Pichel and Pringault, 2001). This ability to follow water is likely an important mechanism for long-term survival of desert soil microbial communities. Soil crusts are damaged by trampling disturbances caused by livestock grazing, tourist activities (e.g., hiking, biking), and off-road vehicle traffic. Such disturbances lead to reduced diversity of crust organisms and increased soil erosion. Seedling germination and establishment are also impacted. Crust recovery can take decades.

Most information on microbial biomass, community composition, and diversity comes from studies of surface soil. The deep subsurface environment, that region below the top few meters of soil that includes deep aquifers, caves, bedrock, and unconsolidated sediments, was once considered hostile to and devoid of living organisms. However, research over the past decade has shown that large numbers of microorganisms reside in the subsurface, often to depths of tens to hundreds of meters. Subsurface microbiology emerged as a discipline largely in response to groundwater quality issues and because recent technological and methodological advances have made possible the characterization of samples collected from deep environments. The field is becoming increasingly interdisciplinary as scientists from other disciplines (e.g., geology, hydrology, geochemistry, and environmental engineering) are becoming interested in understanding the role of microorganisms in soil genesis, contaminant degradation, maintenance of groundwater quality, and the evolution of geological formations (e.g., caves). Additionally, many novel microorganisms with unique biochemical and genetic traits have been isolated from subsurface environments, and these organisms may have important industrial and pharmaceutical uses.

Deep subsurface microbial populations are dominated by bacteria, and nearly all of the major taxonomic and physiological prokaryotic groups have been found. Bacterial densities range from less than $10^1$ to $10^8$ cells per gram of material,

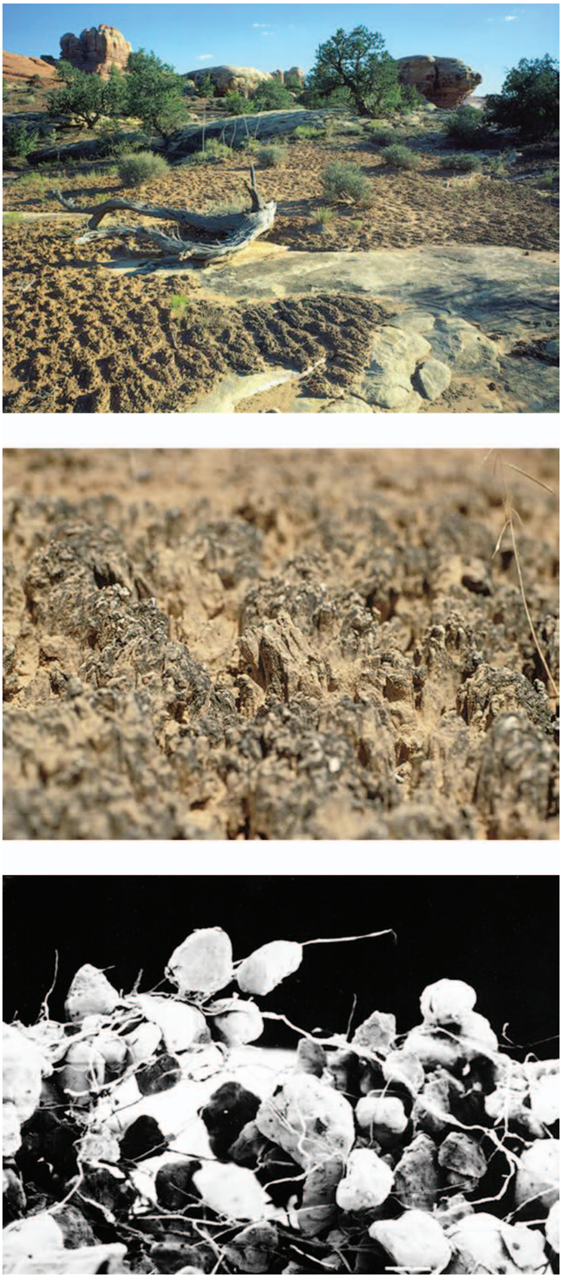


FIGURE 11.7 Biological soil crusts. (Top) Landscape with healthy soil crusts. (Middle) Close-up of mature crusts on the Colorado Plateau, USA. (Bottom) Cyanobacteria adhered to sand grains. (Courtesy of Jayne Belnap, USGS Canyonlands Field Station, Moab, UT, USA. Used by permission.)

TABLE 11.1 Subsurface Bacterial Abundance in Shallow (<10 m) and Deep (>10 m) Vadose Zone Geologic Materials (Adapted from Kieft and Brockman, 2001)

| Depth (m) | Plate counts (cfu $g^{-1}$) | Microscopic counts (cells $g^{-1}$) |
|---|---|---|
| <10 | $<1 \times 10^1$–$8 \times 10^6$ | $7 \times 10^6$–$1 \times 10^8$ |
| >10 | $<1 \times 10^1$–$3 \times 10^7$ | $3 \times 10^4$–$5 \times 10^7$ |

depending on the method of enumeration and the depth of sample collection (Table 11.1). Groundwaters sampled from aquifers or unconsolidated sediments have bacterial concentrations ranging from $10^3$ to $10^6$ cells $ml^{-1}$. Microbial biomass in the deep subsurface is typically several to many orders of magnitude lower than that observed for surface soils. The level of microbial diversity is also much lower in the subsurface compared to surface soils (Zhou *et al.*, 2004). For example, recent analysis of saturated and unsaturated subsurface soils from Virginia and Delaware indicated that molecular sequences of the bacterial community fell into 6 phylogenetic divisions compared to 13 divisions for surface soils (Zhou *et al.*, 2004). Subsurface bacterial communities have a high proportion of nonculturable cells, and metabolic rates are very slow compared to terrestrial surface environments (Fredrickson and Fletcher, 2001). Microbial activity at these depths is limited by water availability, temperature, and availability of energy sources. Energy sources include low-concentration organic substrates or reduced inorganic substrates such as $H_2$, $CH_4$, or $S_2^-$. As in surface soils, the size and interconnectivity of pores is an important factor regulating microbial growth. In the subsurface environment, pores exist in unconsolidated materials or as fractures or fissures in consolidated rock. Significant microbial activity occurs in pores 0.2 to 15 μm in size, whereas little to no activity has been observed in pores with openings of less than 0.2 μm.

While bacteria dominate most subsurface microbial communities, protozoa and fungi have been observed at depth under certain conditions. Protozoan numbers are typically at or below the level of detection at unpolluted sites; however, large protozoan populations have been observed in the subsurface of contaminated sites (Sinclair *et al.*, 1993). Protozoan presence may thus represent a useful indicator of environmental contamination. Protozoa have been shown to stimulate subsurface nitrification (Strauss and Dodds, 1997) and bacterial degradation of dissolved organic C (Kinner *et al.*, 1998). Mycorrhizal hyphae (both ectomycorrhizal and arbuscular mycorrhizal taxa) associated with chaparral plants growing in southern California were recovered in fractured, granitic bedrock at depths greater than 2 m (Egerton-Warburton *et al.*, 2003). The ability of mycorrhiza to grow and function in subsurface environments may enable plants living in dry climates to survive drought conditions by enhancing water and nutrient uptake from bedrock sources.

The study of subsurface microorganisms is transforming our ideas regarding the extent of the biosphere and the role that microorganisms have and continue to play in the evolution of the subsurface environment. As one example, scientists have recently found evidence suggesting that Lower Kane Cave in Wyoming was



FIGURE 11.8 Gypsum associated with biofilms containing sulfur-oxidizing bacteria in Lower Kane Cave, Wyoming, USA. (Photo courtesy Annette Engel, Louisiana State University. Used by permission.)

formed by microbial activity rather than abiotic chemical reactions as was previously thought (Fig. 11.8). Sulfur-oxidizing bacteria colonize carbonate surfaces in the cave, use hydrogen sulfide from a thermal spring as an energy source, and produce sulfuric acid as a by-product (Engel *et al.*, 2004). The microbially produced acid facilitates the conversion of limestone to gypsum, which is subsequently more easily dissolved by water. This work has provided new insights into the role of microorganisms in cave formation and enlargement.

## MICROSCALE HETEROGENEITY IN MICROBIAL POPULATIONS

Intact soil is a continuum of mineral particles, organic materials, pore spaces, and organisms. The shape and arrangement of soil mineral and organic particles are such that a network of pores of various shapes and sizes exists. Between 45 and 60% of the total soil volume comprises pores that are either air or water filled depending on moisture conditions. These pores may be open and connected to adjoining pores or closed and isolated from the surrounding soil. Pores of different shapes, sizes, and degree of continuity provide a mosaic of microbial habitats with very different physical, chemical, and biological characteristics, resulting in an uneven distribution of soil organisms. Since soil organisms themselves vary in size, structural heterogeneity at this scale determines where a particular organism can reside, the degree to which its movement is restricted, and its interactions with other organisms.

The heterogeneous nature of the soil pore network plays a fundamental role in determining microbial abundance, activity, and community composition by affecting the relative proportion of air- versus water-filled pores, which in turn regulates

water and nutrient availability, gas diffusion, and biotic interactions such as competition and predation. Microbial activity, measured as respiratory output (i.e., $CO_2$ evolution), is maximized when about 60% of the total soil pore space is water filled. As soil moisture declines below this level, pores become poorly interconnected, water circulation becomes restricted, and dissolved nutrients, which are carried by the soil solution, become less available for microbial utilization. Soil drying leads to a reduction of microbial biomass, particularly in the larger pores where organisms are subjected to more frequent alterations between desiccation and wetting.

At the other extreme, when most or all of the pores are filled with water, oxygen becomes limiting since diffusion rates are significantly greater in air than through water. Gas diffusion into micropores is particularly slow since small pores often retain water even under dry conditions. Restricted oxygen diffusion into micropores combined with biological oxygen consumption during the decomposition of organic matter can lead to the rapid development and persistence of anaerobic conditions. Thus survival of soil biota residing in small pores depends on their ability to carry out anaerobic respiration (e.g., denitrification), replacing oxygen with an alternative electron acceptor (e.g., $NO_3^-$).

Soil bacteria range in size from small "dwarf" cells ($<0.1\,\mu m^3$) to large cells greater than $0.2\,\mu m^3$. Small cells make up the vast majority of cell numbers ($>80\%$); however, large cells account for most (85–90%) of the soil bacterial biomass (Blackwood and Paul, 2003). Bacteria can occupy both large and small soil pores; however, more than 80% of bacteria are thought to reside preferentially in small pores. The maximum diameter of pores most frequently colonized by bacteria is estimated to range from 2.5 to $9\,\mu m$ for fine- and coarse-textured soils, respectively. Few bacteria have been observed to reside in pores $<0.8\,\mu m$ in diameter, which means that 20–50% of the total soil pore volume, depending on soil texture and the pore size distribution, cannot be accessed and utilized by the microbial community.

Electron microscopy has revealed that bacteria often occur as isolated cells or small colonies ($<10$ cells) associated with decaying organic matter; however, larger colonies of several 100 cells have been observed on the surface of aggregates isolated from a clayey pasture soil and in soils under native vegetation (Fig. 11.9). Bacterial cells are often embedded in mucilage, a sticky substance of bacterial origin to which clay particles attach. Clay encapsulation and residence in small pores may provide bacteria with protection against desiccation, predation, bacteriophage attack, digestion during travel through an earthworm gut, and the deleterious effects of introduced gases such as ethylene bromide, a soil fumigant.

Fungi, protozoa, and algae are found mainly in pores larger than $5\,\mu m$. Fungal hyphae are commonly observed on aggregate surfaces and typically do not enter small microaggregates ($<30\,\mu m$). Like bacteria, fungal hyphae are often sheathed in extracellular mucilage, which not only serves as protection against predation and desiccation, but also is a gluing agent in the soil aggregation process. Mycelial fungi develop extensive hyphal networks and as they grow through the soil and

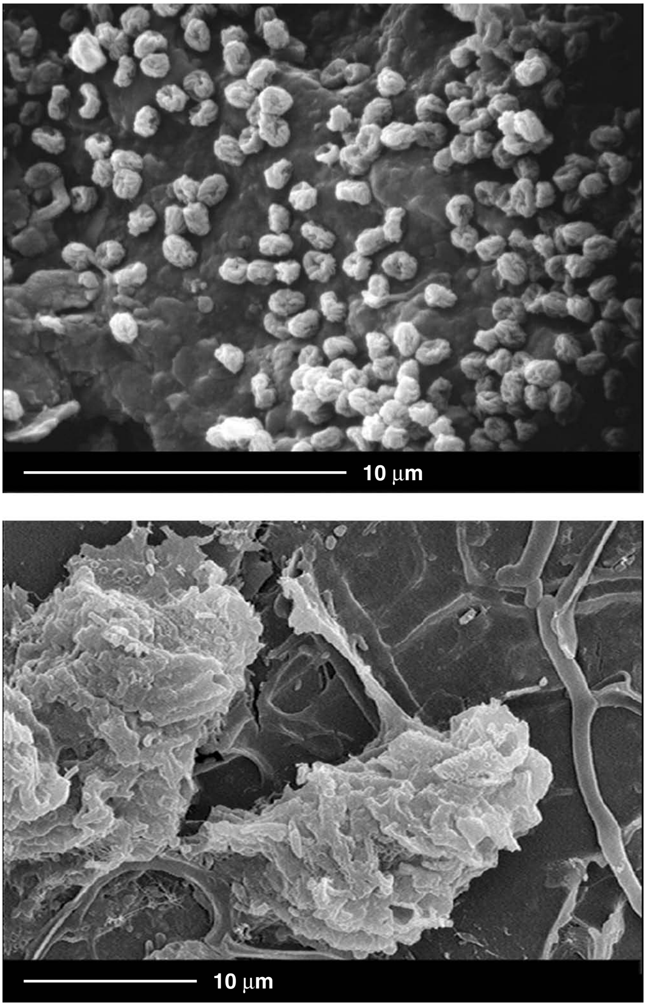



FIGURE 11.9 (Top) Bacteria on the surface of an aggregate isolated from a grassland soil. (Bottom) An amoeba with its extended pseudopodia engulfing bacteria. (Photos courtesy of V. V. S. R. Gupta, CSIRO Land and Water, Glen Osmond, South Australia, Australia. Used by permission.)

over aggregate surfaces, they bind soil particles together, thereby playing an important role in the formation and stabilization of aggregates.

Soil heterogeneity indirectly influences nutrient cycling dynamics by restricting organism movement and thereby modifying the interactions between organisms. For example, small pores influence trophic relationships and nutrient mineralization by providing refuges and protection for smaller organisms, particularly bacteria, against attack from larger predators (e.g., protozoa) that are typically unable to enter smaller pores. The location of bacteria within the pore network is a key factor in their growth and activity. Bacterial populations are consistently high in small pores,

but highly variable in large pores where they are vulnerable to being consumed. This may explain, in part, why introduced bacteria (e.g., *Rhizobium* and biocontrol organisms) often exhibit poor survival relative to indigenous bacteria. When they are introduced in such a way as to be transported by water movement into small, protected pores, their ability to persist is enhanced. This example stresses the importance of considering soil structure and microscale heterogeneity when studying the distribution of soil organisms.

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PART

# IV

# BIOCHEMISTRY AND BIOGEOCHEMISTRY

# 12

# Carbon Cycling and Formation of Soil Organic Matter

William Horwath



## INTRODUCTION

Carbon (C) was deposited on earth from carbonaceous comets and asteroids (Anders, 1989) in both organic and inorganic forms. The extraterrestrial C contained complex compounds including hydrocarbons, organic acids, and amino compounds essential to the evolution of cellular life forms. The "carbon cycle" is the transfer of C among the atmosphere, oceans, land, and life. The C cycle is composed of both long-term and short-term cycles. The subject of this chapter is to understand the short-term C cycle, which includes the terrestrial components of plants, soil biota, and soil organic matter (SOM).

## LONG-TERM CARBON CYCLE

The thermal degradation of comets and asteroids entering the earth's atmosphere was presumed to be the initial source of atmospheric carbon dioxide ($CO_2$). The accumulation of $CO_2$ in the atmosphere resulted in the first major active component of the global C cycle: dissolved carbonates ($CO_3^-$). The weathering of calcium (Ca)–magnesium (Mg)- and silicate (Si)-containing rocks dissolved by carbonic acid ($HCO_3^-$) found in precipitation and other water sources consumed atmospheric $CO_2$ (Fig. 12.1). The following describes this chemical reaction.

$$2CO_2 + 2H_2O + CaSiO_3 \rightarrow Ca^{2+} + 2HCO_3^- + H_4SiO_4.$$

These chemical species, particularly Ca and carbonates, are transported in a dissolved state by rivers to the ocean, where the following reaction occurs:

$$Ca^{2+} + 2HCO_3^- \rightarrow CaCO_3 + CO_2 + H_2O.$$

The overall reaction consumes atmospheric $CO_2$ and transfers it to the near-permanent geologic reservoirs through processes encompassing terrestrial weathering and marine carbonate sedimentation (Berner, 2004). Carbonates formed during pedogenesis, termed caliche, are small in comparison to marine carbonate sedimentation.

The weathering of Ca–Mg Si rocks and sedimentation of marine carbonates is a large sink of atmospheric $CO_2$ (Box 12.1). The uplift of the Himalaya mountains is thought to have increased Ca–Mg Si rock weathering, consuming large amounts of atmospheric $CO_2$. This resulted in the late Cenozoic cooling due to depletion of atmospheric $CO_2$ (Raymo, 1991). The steep oregenic uplift associated with continental formation dynamics enhances primary mineral weathering by increasing

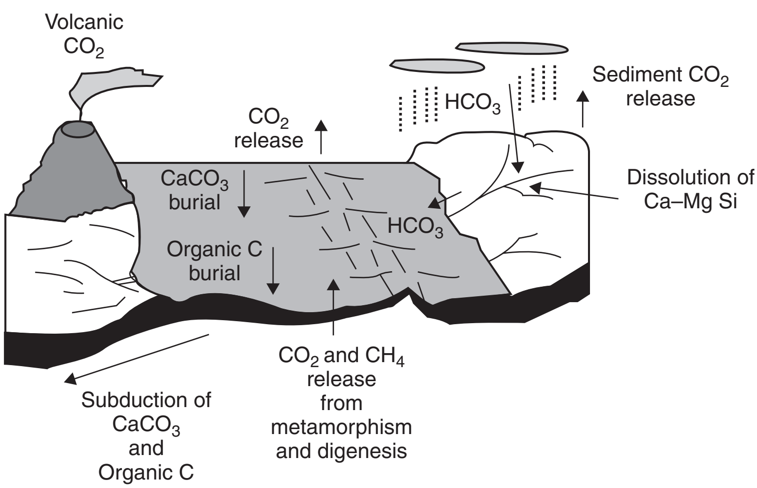



FIGURE 12.1 The long-term C cycle (adapted from Berner, 2004).

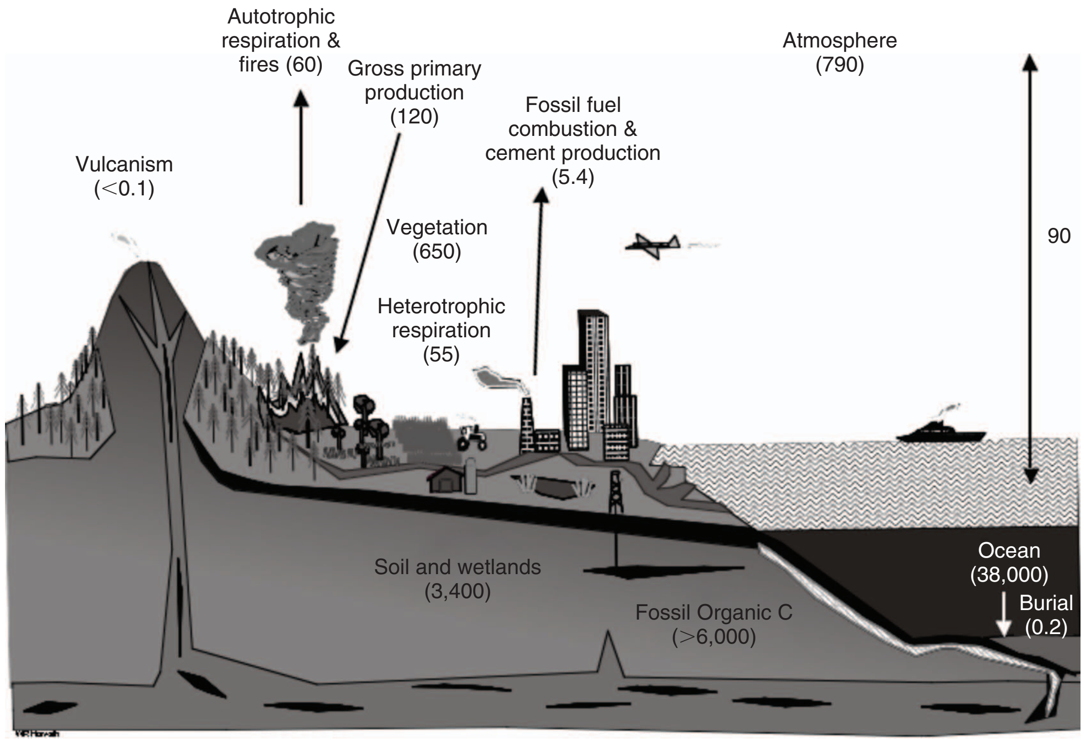
erosive events, constantly exposing Ca–Mg Si rocks to further weathering. Following the subduction of sedimentary rock into the earth's mantle, carbonates eventually undergo thermal weathering and are released as $CO_2$ back to the atmosphere primarily through volcanic activity (Fig. 12.1). This process represented a critical step in controlling atmospheric $CO_2$ content of the atmosphere early in the earth's history.

During the Devonian, the evolution of large vascular plants perturbed the long-term carbonate sedimentation cycle by accelerating the weathering of Si rocks and by removal of $CO_2$ from the atmosphere through burial of organic matter in sediments. Accelerated weathering of silicate rocks occurred as a result of the production of organic acids during growth and decomposition of plant debris. The prolific growth of vascular plants led to the accumulation of large amounts of organic matter, eventually leading to the formation of large fossil fuel deposits so vital to today's society. This period also saw an increase in atmospheric oxygen ($O_2$) from photosynthetic activity and absence of oxidation of buried C-rich organic matter. The evolution of microbial-resistant lignin, a component of the secondary cell wall of vascular plants, is surmised to have contributed to the large quantities of plant debris being deposited. It has been hypothesized that the initial burial of large amounts of lignaceous plant material occurred as a result of the absence of lignin-degrading organisms, which evolved later in the Paleozoic

### BOX 12.1

Terrestrial photosynthesis and decomposition are closely balanced. On a geological time scale photosynthesis has been slightly larger than decomposition, leading to reserves of fossil fuels that are four times as great as the C stored in the soil. Oceanic photosynthesis and decay are approximately equal to that of terrestrial, but this is carried out by biota of only 45 petagrams (Pg), resulting in a much faster turnover rate than in the terrestrial environment. A petagram is equivalent to $10^{15}$ g or $10^9$ metric tons. The oceans have large reserves of dissolved C, both inorganic and organic, especially in the thermocline and in the deep sea, representing a pool of 38,000 Pg C, substantially larger than soil C. Woody components of the land biota account for 75% of stored terrestrial plant C. Subtracting the values for respiration from gross primary production gives net primary production values of 50–60 Pg, indicating that it takes approximately 10 years for the terrestrial plants to recycle their C. This represents, on average, fast turnover rates for leachates and easily decomposable residues relative to the tens, hundreds, and even thousands of years necessary for the turnover of woody components and humified material and hundreds of thousands of years for deep ocean and fossil C (Houghton *et al.*, 2001).

BOX 12.1 (CONTINUED)



(Robinson, 1990). This may be why the Carboniferous and Permian coal reserves outweigh those from any other period in the earth's history.

Combined, the carbonate–Si and organic matter cycles dominate controls on the long-term levels of $CO_2$ and $O_2$ over the millions of years of the Phanerozoic eon. These dominant C cycles were probably in play during the Precambrian, but the fossil record, geology, and climate data are not as abundant as for the Phanerozoic, making the interpretation of the Precambrian C cycle more difficult. Organic matter deposition today occurs more slowly than during the Devonian, presumably because of less primary production and the evolution of plant-debris-degrading organisms. This, coupled with reduced volcanic activity, represents the new equilibrium of atmospheric $CO_2$ controlled by these cycles. During the past 500,000 years (Box 12.2), the atmospheric $CO_2$ level has been closely related to the advance and retreat of glaciers and was lower than the present-day level of 370 $ppm_V$ (Petit *et al.*, 1999). In the past 30,000 to 40,000 years, $CO_2$ levels of 200 $ppm_V$ or less were common. Carbon dioxide levels exceeding 1000 $ppm_V$ were common prior to this period up to 4 million years before present (Retallack, 2001). Though these cycles are robust, it is evident that the burning of fossils fuels can have an immediate impact on the C cycle that has led to accelerated changes in the cycling of C at the global scale.

## THE SHORT-TERM C CYCLE

The short-term C cycle is dominated by the interplay of terrestrial and marine photosynthesis, respiration, and organic matter formation (Fig. 12.2). The short-term C cycle is dependent on two principle gases, $CO_2$ and methane ($CH_4$). Perturbations of the short-term C cycle causing changes in the concentration of these two gases in the atmosphere cause potential changes in climate because both are greenhouse gases. These gases absorb outgoing infrared radiation from the earth's surface, trapping heat. Over time, these gases have regulated the temperature of the planet. Other greenhouse gases produced by microbes such as nitrous oxide also play an important role in climate change (see Chap. 13). Variations in the sun's energy output and changes in earth's distance from the sun have also contributed to climate change over the Quaternary.

It has been estimated that in the year 1860 the atmosphere contained about 260 ppm $CO_2$; in 2006 it contained approximately 375 $ppm_V$ or 765 Pg C (Box 12.2). Since the late 1800s, fossil fuel use, forest clearing, and the conversion of extensive areas of virgin land to agriculture have led to a net transfer of terrestrial C to the atmosphere (Falkowski *et al.*, 2000). This has been partially offset by the continuous net uptake of carbonates as sedimentary rocks in the oceans. Approximately up to 20% of the C evolved in the last 100 years cannot be accounted for in present estimates of the global C budget. Suggestions have been made that terrestrial C sequestration potential of terrestrial ecosystems has been underestimated. This may be a result of higher photosynthetic rates and plant water use efficiency under

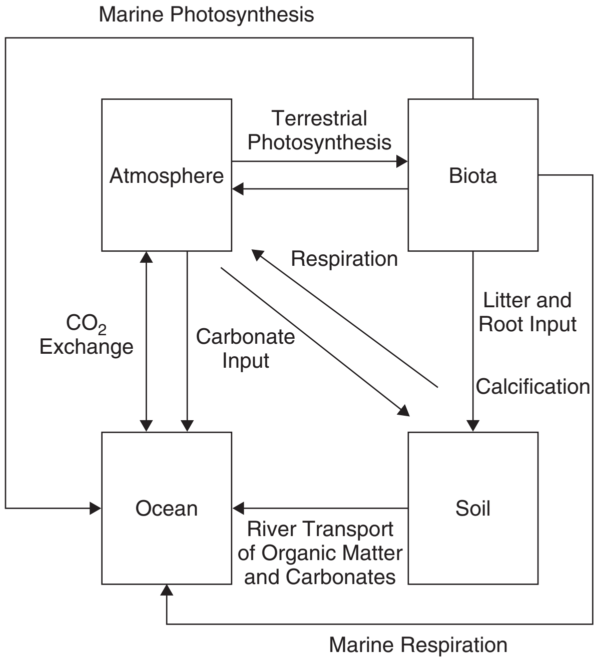
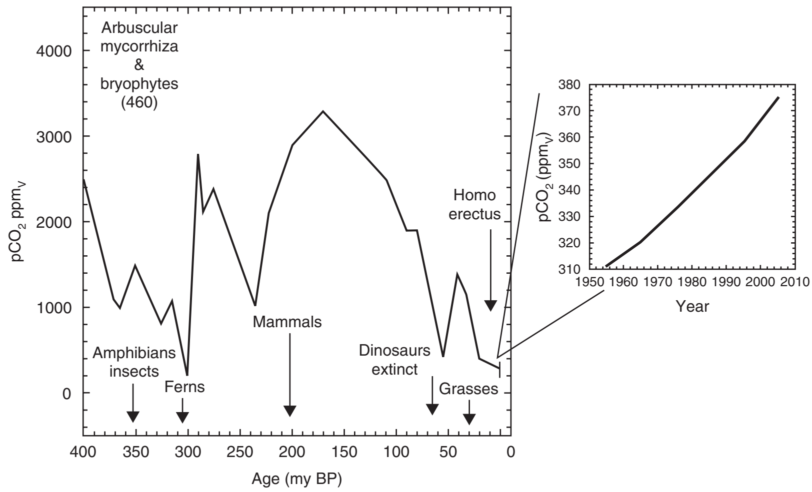



FIGURE 12.2 The short-term C cycle (adapted from Berner, 2004).

### BOX 12.2

The atmospheric $CO_2$ level has fluctuated wildly over the past 400 million years. The variation can be attributed to changes in gross primary production caused by such events as volcanism and changes in the orientation of the earth's axis that affected the climate. Over the past 200 years, as atmospheric $CO_2$ has risen by over 100 $ppm_V$, plants have responded by decreasing stomatal density. The inverse relationship between atmospheric $CO_2$ concentration and stomatal density has been used as a paleobarometer to estimate past atmospheric $CO_2$ concentration. The evolution of trees provides an excellent fossil record of leaf structures, specifically stomata and epidermal cells. The environmental factors affecting the interpretation of stomatal density are minimized by including the relationship of stomatal index (percentage stomates over stomates plus epidermal cells). Retallack (2001) used the genus *Ginkgo*, with a fossil record dating to the late Triassic period (229 Mya), and extended the record to the Permian using the stomatal index of plant species with morphologies similar to that of *Ginkgo*.

### Box 12.2 (Continued)




increasing atmospheric $CO_2$. Extensive areas of cultivated land have recently been returned to trees and grasses promoting soil C sequestration. Nitrogen deposition from anthropogenic activities has increased plant production. Increases in rice production using flooded paddies and animal husbandry have contributed to increased $CH_4$ emissions. These biological processes combined with considerable uncertainty in the gross rates of Ca and Mg dissolution from terrestrial sources may explain discrepancies in accounting in the global C budget.

## Ecosystem C Cycling

Photosynthesis converts inorganic C ($CO_2$) into organic C through gross primary production (GPP) (Fig. 12.3). Some of this carbon is returned to the atmosphere as plant-respired $CO_2$; the remainder becomes plant biomass and is termed net primary production (NPP). Free-living autotrophic microbes, such as algae, also contribute to GPP and NPP. Net secondary production (NSP) is the consumption of NPP by fauna and microorganisms. The standing stock of C in an ecosystem is defined as GPP less the respiratory loss of autotrophs (photosynthesizers) and decomposers (heterotrophs) and is termed net ecosystem production (NEP). Free-living microorganisms and soil fauna consume (decompose) the majority of NPP. The process of decomposition occurs on the order of days to decades and is dependent on environmental conditions and the quality of the plant material entering

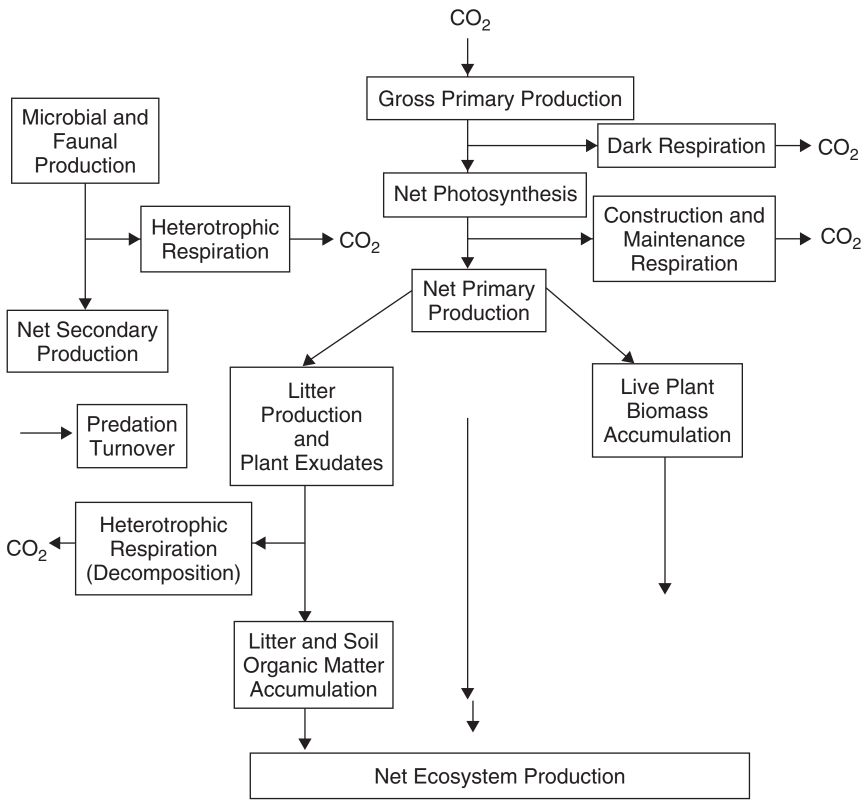



FIGURE 12.3 Various components of gross primary production and net ecosystem production.

the soil. The selective preservation of some resistant plant constituents, such as lignin, and the activity of microorganisms produce precursors to humic substances described later in this chapter. Soil humic substances can persist for thousands of years and are important stable C pools making up two-thirds of terrestrial C stores. GPP, NPP, NSP, and NEP are processes usually measured in terms of g C $m^{-2}$ $year^{-1}$ or similar units.

The NEP of different ecosystems varies considerably depending on plant species, soil type, and climate. Gross primary production is often controlled by the ability of soil biota to release through decomposition essential nutrients such as N and P to sustain NPP. Figure 12.4 shows the size of various plant, microbial, and soil C and N pools in a hybrid poplar system. The activity of decomposers is required to release a small portion of the large soil N pool for NEP. Forests, including tropical, temperate, and boreal forests, have the largest NEP due to the accumulation of wood. Humid tropical ecosystems in general have the highest GPP of all terrestrial environments despite having a limited essential nutrient pool. They are characterized by high production and decomposition rates, where nutrients are

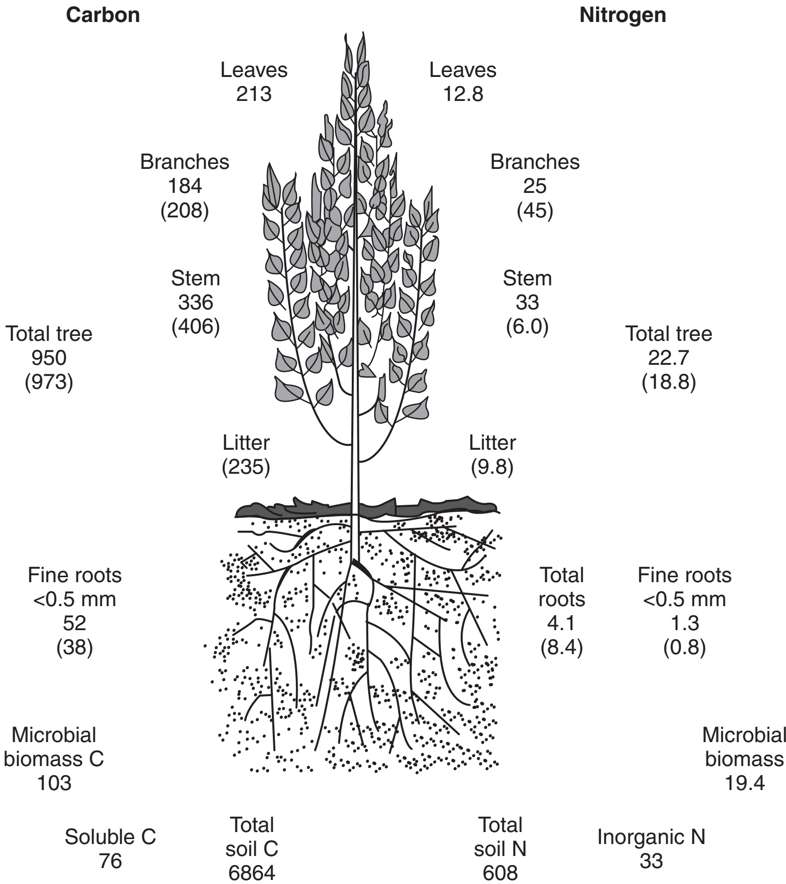



FIGURE 12.4 The distribution of C and N in a 2-year hybrid poplar stand during July. Microbial N is similar to poplar N, showing the importance of the microbial biomass in influencing nutrient availability. The values in parentheses represent C and N amounts for November.

actively cycling through plant litter and soil biota, and less storage in soil as organic matter. Optimal temperature and moisture conditions for decomposition prevail in humid tropical environments, leading to relatively closed nutrient cycles in the absence of disturbance. In northern latitude ecosystems, lower temperatures lead to slower decomposition of plant litter. The accumulation of plant matter in forest floor litter layers can withhold from the soil solution essential nutrients, particularly N. Disturbance such as fire, a physical oxidative decomposition process, is often required to release nutrients to increase NPP. Plant community succession also leads to changes in nutrient cycling through changes in the quantity and quality of litter inputs or the nutrient requirements of succeeding plant and microbial communities.

## COMPOSITION AND TURNOVER OF C INPUTS TO SOIL

The quantity and quality of plant litter and microbial turnover to soil greatly influence the outcome of decomposition processes. The sizes of the standing leaf and microbial N pools are similar in most ecosystems, indicating the importance of soil organisms for influencing available N for plant uptake. Various biological constituents such as cytoplasm and cell wall material of plant, microbial, and faunal inputs decompose at vastly different rates (see Chap. 16 for more details on turnover). Decomposition models often relate the decomposability of soil inputs to their C to N ratio, to their N content, and to the amount of resistant material such as lignin or chitin that they contain. Other factors such as their polyphenol (a secondary plant metabolite) content may impact decomposition through phytotoxic interactions. The availability of nutrients in soil solution affects not only the quality and quantity of inputs but also the ability of decomposers to consume the inputs. Higher decomposition rates result from increases in available N ($NH_4^+$ and $NO_3^-$), which causes vegetative growth with low C:N ratio (high N content). Elevated $CO_2$ can produce plant litter with a higher C:N ratio, making it more difficult to decompose.

Net primary production adds to soil litter or detritus composed of leaves, branches, reproductive structures, leachates, and belowground products such as roots, exudates, and sloughed root cells. Plants contain an array of cytoplasm and cell wall components that require a complex scheme of enzymes to decompose. Unique plant components include cellulose, hemicelluloses, phenylpropanoids (lignin), and polyphenols (tannins). Microorganisms produce unique cell wall structures (e.g., peptidoglycans) and pigments (e.g., melanin). Leachate C is generally more important in forest systems in which significant leaching of dissolved organic C from the canopy and litter layer can occur. Root exudates are low-molecular-weight carbohydrates and amino-type compounds (amino acids, amino sugars, and small peptides) typically exuded from roots and mycorrhiza. The turnover of the NPP and NSP is an important source of humic substances that contribute to SOM formation and maintenance.

The various components of plant inputs to soil vary greatly as a source of energy and nutrients for NSP. The majority of plant inputs are C polymers (e.g., cellulose) and hydrocarbons (e.g., lignin) that contain few essential nutrients to facilitate decomposition. Cytoplasmic constituents such as sugars, amino compounds. and organic acids comprise up to 10% of plant residue dry weight (Table 12.1). Cytoplasm proteins contain the majority of N in plant tissue. Proteins and peptides are hydrolyzed by proteases and peptidases to individual amino acids during decomposition (Fig. 12.5). These labile N sources and other C-containing compounds provide the initial energy source and nutrients to start the decomposition process. Proteins also contain significant amounts of S in the form of the amino acids cystine and methionine. The protein content of plant tissues ranges from 1% in cell walls to 22% in meristematic regions and seeds. In leaves,

TABLE 12.1 Percentage of Cytoplasmic and Cell Wall Components in Plants (Adapted from Horwath, 2002)

| Plant component | % of total |
|---|---|
| Waxes and pigment | 1 |
| Amino acids, sugars, nucleotides, etc. | 5 |
| Starch | 2–20 |
| Protein | 5–7 |
| Hemicellulose | 15–20 |
| Cellulose | 4–50 |
| Lignin | 8–20 |
| Secondary compounds | 2–30 |

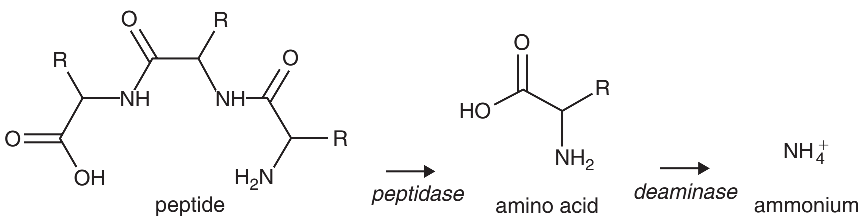



FIGURE 12.5 Breakdown of protein/polypeptides into amino acids and further mineralization to ammonium is a source of N for decomposition, microbial growth, and plant uptake.

the photosynthetic enzyme ribulose bisphosphatase can contain up to 70% of the total tissue N.

Hemicellulose and cellulose are important cell wall polymers composing 10 to 70% of plant residues. The secondary cell wall contains from 5 to 30% lignin, a unique terrestrial plant hydrocarbon that provides a rigid exoskeleton and defense against pathogens. Extractable phenols and tannins are a significant component of some plants, especially in forest systems, and comprise up to 30% of their dry weight. These complex compounds are believed to be deterrents to herbivores and their presence can alter C and N mineralization dynamics dramatically. During later stages of decomposition, when low-N cellulose and lignin are being metabolized, soil nutrients from SOM and mineral, ion-exchange sites are used to metabolize the C compounds in plant litter.

Examination of common, $G^-$ prokaryotic bacteria such as *Escherichia coli* reveals a variety of C compounds, some similar to plants and others that are vastly different (Table 12.2). Protein makes up 55% of the cell dry weight of this organism. RNA is the next largest N component at 20.5% of its dry weight. The majority (80%) of this is ribosomal RNA (23S, 16S, and 5S RNA). Fungi have similar structures of slightly larger size, e.g., 18S and 25S RNA. Transfer RNA constitutes 15% and messenger RNA 4% of the total RNA. DNA accounts for 3% of the bacterial cell dry weight. Thus the N-containing genetic constituents, such as guanine, adenine, cytosine, and thymine and to some extent uracil, form a significant (24%) source

TABLE 12.2 Biochemical and Molecular Composition of Prokaryotic Cells[a]

| Molecule | Dry weight(%)[b] | Molecules per cell | Different kinds of molecules |
|---|---|---|---|
| Total macromolecules | 96 | 24,610,000 | 2500 |
| Protein | 55 | 2,350,000 | 1850 |
| Polysaccharide | 5 | 4,300 | 2 |
| Lipid | 9.1 | 22,000,000 | 4 |
| Lipopolysaccharide | 3.4 | 1,430,000 | 1 |
| DNA | 3.1 | 2 | 1 |
| RNA | 20.5 | 255,500 | 660 |
| Murein | 2.5 | 1 | 1 |
| Glycogen | 2.5 | 4,360 | 1 |
| Total monomers | 3.0 | —[c] | 350 |
| Amino acids | 0.5 | — | 100 |
| Sugars | 3.0 | — | 50 |
| Nucleotides | 2.0 | — | 200 |
| Inorganic | 1.0 | — | 18 |
| Total | 100% | | |

[a]Data from Neidhardt *et al.* (1996).
[b]Dry weight calculated on basis of 70% cell water content.
[c]Reliable estimates are lacking.

of potentially mineralizable N. Soil fungi often concentrate their cytoplasm and active metabolic constituents at their growing tips. In soil, they have been shown to have only one-tenth as much DNA per unit weight as bacteria and generally have higher C:N ratios.

Bacteria have C:N ratios that range from 3.5:1 to 7:1, while fungi range from 10:1 to 15:1. The average total microbial biomass has a C:N ratio of 4:1 to 8:1. The C:N ratio depends on the broad groups of bacteria and fungi present. The microbial C:N ratio as determined by physiological methods can be a crude measure of microbial diversity. Bacteria often dominate highly disturbed systems such as agricultural fields. Other ecosystems where bacteria flourish are wet meadows, marshes, and wet tropical forests. Fungi tend to dominate in soils that are well aerated and less disturbed, such as grasslands, forests, and reduced-tillage agricultural systems.

Microbial growth reflects the dominant groups of organisms present in a soil. The importance of microbial products for forming and maintaining SOM has been shown through the addition of glucose to soil, which produces microbial products that are ultimately more stable than some plant-derived compounds. The biochemical composition of microbial cells varies significantly among groups of microbes (such as fungi, $G^+$ bacteria, $G^-$ bacteria, and actinomycetes). Microbial products such as phenols, amino sugars, and fungal melanins may contribute significantly to maintaining SOM. Information on the composition of the microbial community can be used to improve the process-based C cycling models described in Chap. 16 and provide more insight into humification processes.

## PLANT AND MICROBIAL LIPIDS

Lipids enter the soil from the turnover of plant residues, fauna, and microorganisms. They represent a diverse class of compounds ranging from simple fatty acids to complex sterols, phospholipids, chlorophyll, waxes, and resins (cutins and suberins). Lipids are measured by sequential extraction with nonpolar solvents, such as hexane and chloroform. The average lipid content of most plants is about 5% of dry weight, with leaves containing the greatest amount. The lipid content depends highly on the plant species. The lipid content of high, cutin-containing plants, such as conifers and succulents, may reach 10% or more of the dry weight. The durability of lipids depends on their chemical complexity. Long-chain aliphatic fatty acids and phospholipids, component of membranes, are degraded relatively quickly depending on the degree of saturation or double-bond content. More complex resins can be very recalcitrant and form some of the most decomposition-resistant substances in soil. The hydrophobic character of resins allows them to sorb into hydrophobic domains of SOM, shielding them from enzymatic attack.

Microbial lipids, consisting of more than 500 different types of fatty acids, are similar in function to those of plants and animals but differ structurally. Lipids are present in appreciable amounts in fungal spores and hyphae. The lipid content of fungal mycelium averages 17% by weight and ranges from 1 to 55% depending on the species. Various phospholipids are unique and are useful for identifying microorganisms (see Chap. 3). Assays of specific fungal lipids such as ergosterol have proved useful for quantifying their biomass and for qualitative evidence of their diversity. The bacterial cell is typically 9% lipid (Table 12.2). Microbial lipids degrade readily in their unprotected state, but like plant lipids, once sorbed into the hydrophobic domains of SOM, become protected from further decomposition.

Lipids can accumulate in acidic soils where they can constitute 30% of the total SOM C (Stevenson, 1994). Soil disturbance such as tillage, erosion, or fire is needed to release these compounds into the decomposition cycle. High organic matter soils contain the greatest amount of lipids. Clay soils have higher lipid content than coarse textured or sandy soils. The decomposition of more complex lipids, such as sterols and cutins, requires multifaceted enzymatic systems or groups of enzymes. Cutin is a polymer network of oxygenated C-16 and C-18 fatty acids cross-linked by ester bonds. Gaining attention are compounds accumulating in soils with behavior similar to that of lipids termed polyaromatic hydrocarbons (PAH), released from fossil fuel combustion. Enzymes and degradative processes of the more recalcitrant lipid substances and PAH are not well understood.

## STARCH

Starch is a plant polymer synthesized from glucose and stored in plastids as grains of 1 to 100 nm in diameter (see Fig. 12.6 for glucose structure). It consists

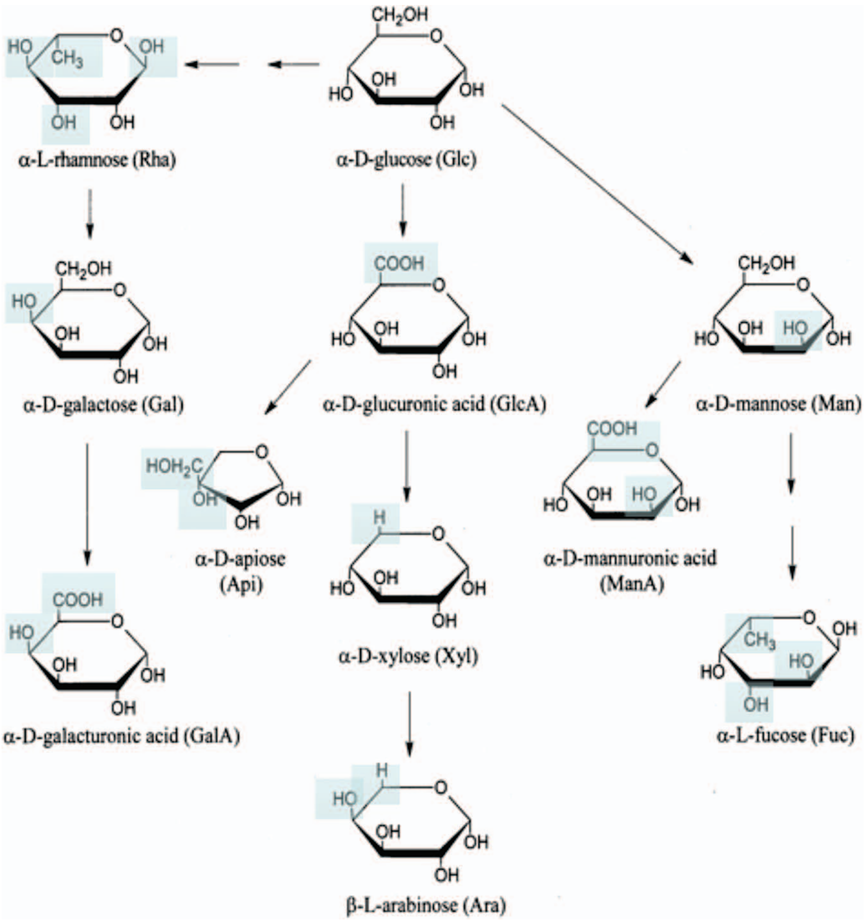



FIGURE 12.6 Common sugars of the plant cell wall and their interconversions. The modifications to convert D-glucose into other sugars are highlighted in light blue.

of two glucose polymers, amylose and amylopectin. Amylose contains long unbranched chains of (1-4)-glucose units. For most plants, amylose can account for up to 30% of the total starch. Amylopectin has a similar structure, linked every 20 to 30 glucose residues by (1-6)-glucose bonds (Fig. 12.7). A class of enzymes known as amylases degrades starch readily. $\alpha$-Amylase catalyzes the cleavage of glucosyl bonds, producing small glucans called dextrins along with glucose and maltose. $\beta$-Amylase cleaves maltose residues from the nonreducing end of the starch molecule. The final degradation of maltose, short-chained glucans and dextrins to glucose, is achieved by $\beta$-glycosidase. Many bacteria and fungi produce these enzymes to convert insoluble starch effectively into easily metabolized glucose.

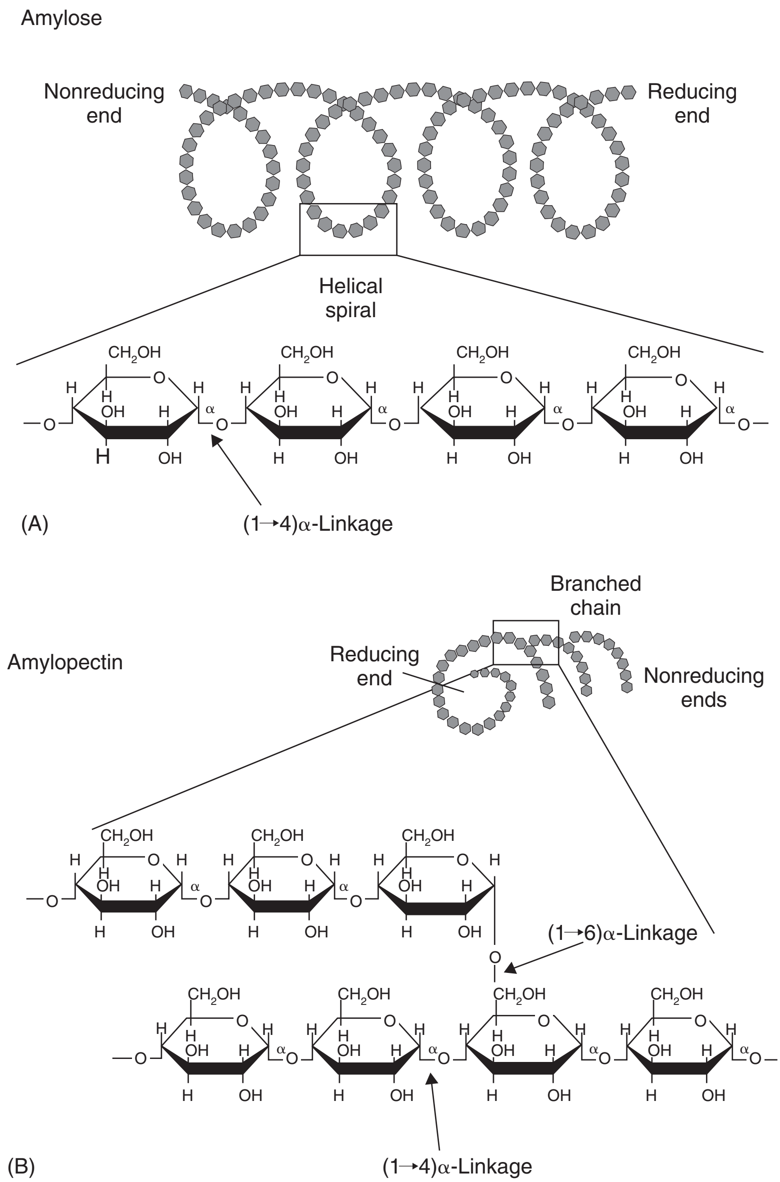



FIGURE 12.7 The structure of starch showing the (A) α(1-4) linkage of amylose and (B) α(1-6) branched linkage of amylopectin.

## HEMICELLULOSES, PECTINS, AND CELLULOSE

The majority of plant carbohydrates are found as polysaccharides as part of the cytoskeleton in the primary and secondary plant cell wall. Polysaccharides are

long chains of sugars that are covalently linked through H bonds. The vast majority are aldoses with the empirical formula $(CH_2O)_n$. All of the monosaccharides in the plant cell wall are derived from glucose and upon alteration form a variety of 5- and 6-C sugars (Fig. 12.6). The sugars are locked into polymerized chains by pyranose or furanose rings to form either hemicellulose/pectic or cellulose microfibrils.

Hemicelluloses and pectin are composed of 5-C sugars or glycans. The majority of glycans of flowering plants consist of D-xylose, D-glucuronic acid, and D-arabinose. A common cross-linked structure is (1-4)-D-glucan and (1-4)-D-xylose found in all dicotyledons and about half of monocots. In many grasses, the major cross-linked glycan is glucuronoarabinoxylan. Cereals and grasses also contain a mixed linkage of 1-3(1-4)-D-glucans as a distinguishing component of the cross-linked glycan–cellulose microfibril network. A mixture of heterogeneous, branched, highly hydrated polysaccharides composed mainly of D-galacturonic acid is called pectin. Pectins are thought to influence cell wall porosity, pH, and cell-to-cell adhesion. Hemicelluloses are reduced to simple sugars by several enzymes known collectively as pectinases. For this reason, the degradation of pectin and hemicellulose is thought to occur together. The study of hemicellulose/pectin degradation has received much attention because of its importance as a point of attack by pathogens and as a means for symbionts such as rhizobia and mycorrhiza to gain access to the middle lamella.

The pectinase enzyme system includes three stages of substrate degradation. The degradation of hemicellulose/pectin is similar to cellulose degradation except that more enzymes are involved. The decoupling of the hemicellulose and pectin cross-linked glycan structure is thought to provide access for other cell wall-degrading enzymes. The release of oligogalacturonides and simple sugars represses the enzyme system and is thought to control microbial succession during litter decomposition. Pectinolytic soil bacteria include species of *Erwinia*, *Arthobacter*, *Pseudomonas*, and *Bacillus.* Common pectinolytic fungi are species of *Aspergillus*, *Rhyzopus*, *Fusarium*, *Sclerotina*, and *Penicillium.* Yeast such as *Conida* and *Kluyveromyces* also exhibit pectinase activity.

Cellulose is the most abundant plant polysaccharide and accounts for 15 to 30% of the primary cell wall dry mass and a greater percentage of the secondary cell wall, especially of woody species. It consists of glucose units linked by $\beta$(1-4) bonds to form D-glucan chains (Fig. 12.8). The chains are cross-linked by H bonds to form paracrystalline assemblages called microfibrils. The average microfibril is composed of 36 individual glucan chains and several thousand individual glucan molecules to reach a length of 2 to 3 mm. Cellulose microfibrils are cross-linked into a network or scaffold with glycans or hemicellulose. In addition to cellulose, plants can synthesize callose by linking glucans in the $\beta$(1-3) configuration similar to that found in yeast and fungi. Callose found in phloem sieve plates, pollen tubes, cotton fibers, and other specialized cells is produced in response to wounding such as fungal hypha penetration of the primary cell wall.

Cellulose microfibrils are decomposed by the enzyme system termed cellulase, composed of endoglucanase, exoglucanase, and $\beta$-glucosidase (also known

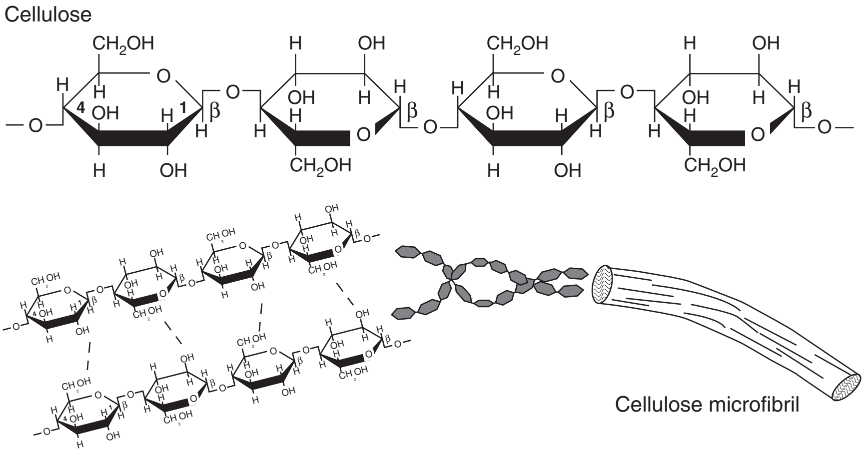



FIGURE 12.8 Cellulose structure showing $\beta$(1-4) linkages combining glucose residues into a single chain polymer. Chains are cross-linked by hydrogen bonds to form cellulose microfibrils.

collectively as cellobiases). The cellulase enzymes have distinct roles in cleaving the various bonds within the microfibril structure. This causes disruption of the crystalline structure followed by depolymerization into short glucose chains. Endoglucanases act randomly on both soluble and insoluble glucose chains by cleaving the $\beta$(1-4) linkages, yielding glucose and cellooligosaccharides. Following this, exoglucanases, including glucanhydrolase, act on the nonreducing ends of the cellulose chains, yielding glucose and cellobiose (glucose dimers) and cellotriose (glucose trimers). In the final step of decomposition, $\beta$-glucosidase hydrolyzes the glucose chain fragments to glucose. Breakdown of the glucose subunits occurs rapidly and the products can inhibit the activity of the cellulase system.

A wide range of organisms degrade cellulose, but only a few have demonstrated the complete depolymerization and hydrolysis of the crystalline microfibril structure *in vitro*. The cellulase system of the fungus genus *Trichoderma* has been extensively studied and shows a large production of endo-$\beta$-glucanases and exo-$\beta$-glucanases but low levels of $\beta$-glucocidases. In contrast, *Aspergillus* produces large amounts of endo-$\beta$-glucanase and $\beta$-glucocidases but low levels of exo-$\beta$-glucanases. *Chaetoium*, an ascomycetous fungus, is found on a wide variety of cellulose materials from paper to composts, especially in moist environments. It produces thermotolerant cellulases that may be commercially viable for converting cellulose to simple sugars from a variety of sources suitable as sources of biofuels. Other fungi with extensively studied cellulase systems include *Cremonium cellulolyticus*, *Penicillium*, *Fusarium*, and the edible mushroom *Agaricus*.

Bacteria have less extensive cellulase systems compared to fungi. Bacterial cellulases are organized into a globular scaffolding protein called cellusomes, bound to their cell walls. These structures coordinate the cellulase system for direct attack of crystalline microfibrils, increasing the activity or efficiency of the individual

enzymes and allowing the bacteria a better chance to ingest the freed glucose. This is in contrast to fungi that secrete cellulases, resulting in the degradation of cellulytic substances away from the hyphae and thus not guaranteeing that the soluble glucose products are ingested. Common aerobic soil bacteria that depolymerize cellulose are *Cellulomas*, *Cellovibrio*, *Pseudomonas*, and *Bacillus.* Anaerobic bacteria include *Acetobacter*, *Bacteriodes*, *Clostridium*, *Fibrobacter*, and *Rummococcus.*

## LIGNIN

After cellulose, lignin is the second most abundant organic substance produced during NPP. Lignin is a complex and dense amorphous secondary cell wall polymer found in the trachea elements and sclerenchyma of terrestrial plants. The basic structure of lignin is based on the phenyl propanoid unit, consisting of an aromatic ring and a 3-C side chain (Fig. 12.9). The conversion of phenylalanine and tyrosine in the shikimic and phenylpropanoid pathways to *p*-coumaric acid by ammonia lyase is the starting point of the phenylpropanoid metabolic pathway that forms the monolignol precursors synapyl, coniferyl, and coumaryl alcohols. Lignin synthesis begins with phenoxy radical coupling, a random self-replicating

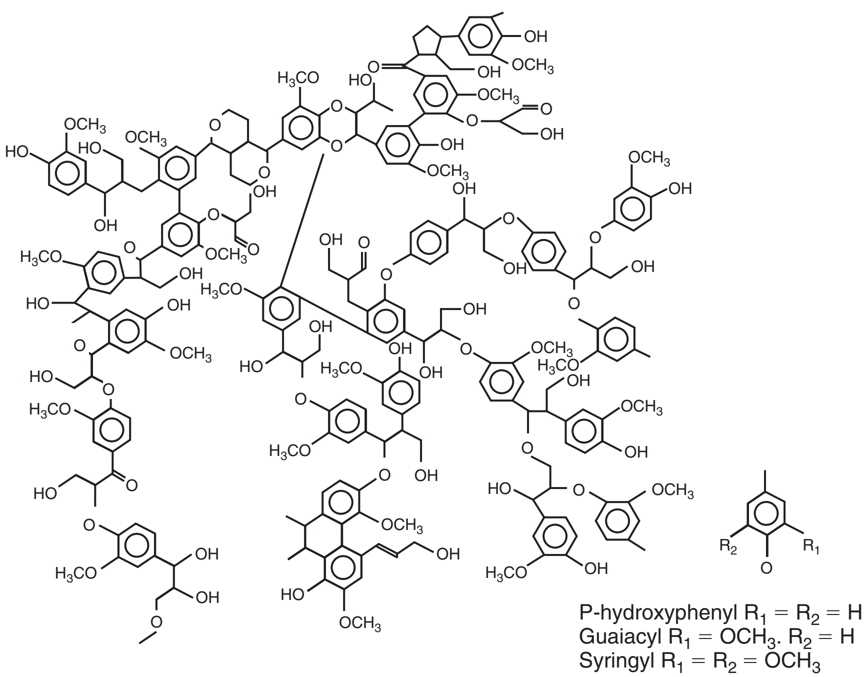



FIGURE 12.9 Proposed structure of a softwood lignin showing the large hydrocarbon content that among other characteristics leads to the hydrophobic character of the plant secondary cell wall.

polymerization, of the monolignols. After polymerization, the lignin monolignol subunits are referred to as *p*-hydroxyphenol, guaiacyl, and syringyl residues, respectively. The synthesis of lignin involves the deposition of monolignols onto a protein template to create an amorphous polymer structure. The random assembly of hydrocarbons is hydrophobic in nature and provides structural rigidity and a barrier against pests and pathogens. In most dicots, the lignin structure contains guaiacyl and syringyl residues. Grass lignins also contain *p*-hydroxyphenol in small amounts. Lignin is thought to be cross-linked to hemicellulose via a cell wall protein called extensin (Fig. 12.10). The actual details of lignification are poorly understood. This has contributed to the incomplete understanding of lignin decomposition.

The dense nature, hydrophobicity, and nonspecific structure of lignin make it difficult for enzymes to attack. It is thought that lignin must be broken into smaller fragments before extensive anaerobic decomposition will proceed. Lignin depolymerization produces a water-soluble, acid-precipitable product not unlike soil humic acids. The gram-negative aerobic bacteria *Pseudomonadaceae*, *Azotobacter*, and *Neisseriaceae* and common actinomycetes *Nocardia* and *Streptomyces* can degrade lignin but not to the same extent as fungi. Whether bacteria can cause complete decomposition and use any lignin C for growth has not been well established. Bacteria may attack parts of the lignin structure to remove the barrier shielding energy-rich cellulose and hemicellulose. Bacteria found in the guts of ruminants and some arthropods also have limited ability to degrade lignin. Lignin is solubilized in termites by gut-inhabiting streptomycetes to liberate the energy-rich cellulose and hemicellulose. The effect of the high pH (9–11) in termite foreguts and the proportion of true lignase activity relative to depolymerization and by-product formation are not known.

Fungi are the most efficient lignin degraders in nature, playing a key role in biotic C cycling. Fungal species that degrade lignin are often grouped into soft rot, brown rot, and white rot fungi based on the color of the decaying substrate. Various fungi represented by Imperfecti and Ascomycetes cause the soft rot of wood. Soft

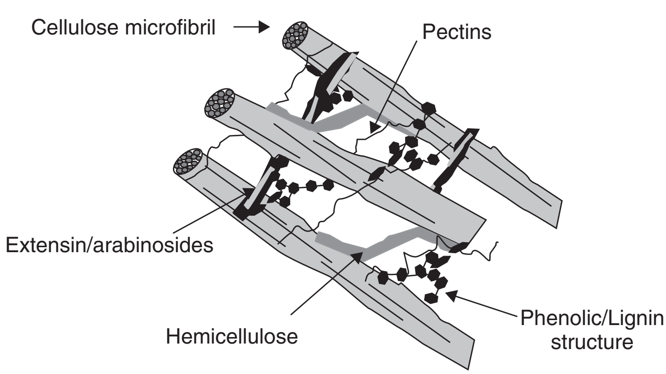



FIGURE 12.10 A three-dimensional model of the secondary cell wall of plants. The model allows visualization of interactions among petic substances, hemicellulose, cellulose, lignin, and extensin.

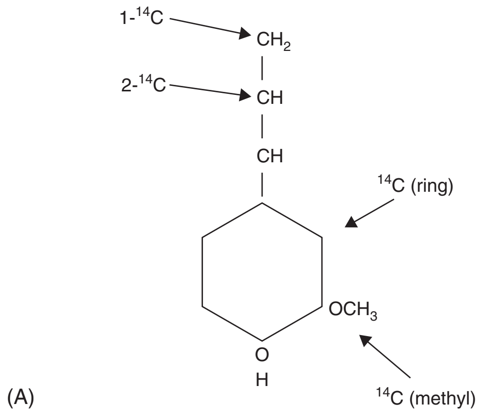
rots prefer polysaccharides but have the ability to remove methylated side chains (R–O–$CH_3$) and cleave aromatic rings, but cannot completely degrade the lignin structure. The soft rot fungi are important in mesic environments and appear to degrade hardwood lignin more effectively than that of softwoods, with *Chaetomium* and *Preussia* being representative organisms. Box 12.3 shows the vulnerability to degradation of C found in specific positions in the monolignol coniferyl alcohol over a 2-year period.

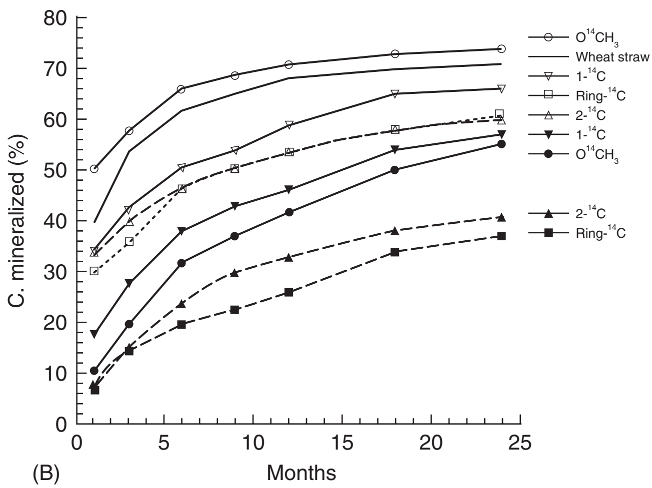
The majority of wood decay is performed by brown and white rot fungi from the basidiomycetes. Brown rots lack ring-cleaving enzymes but readily degrade hemicellulose and cellulose of intact wood. They modify and degrade lignin through demethylation and removal of methylated side chains producing hydroxylated phenols. The oxidation of the lignin aromatic structures causes a characteristic brown color. The separation of polysaccharides from lignin is thought to occur through nonenzymatic oxidation via the production of low-molecular-weight

## BOX 12.3

Radioactive or stable isotopes of C have been used extensively to study lignocellulose decomposition. Isotopically labeled lignocellulose preparations are prepared either chemically or by feeding plants with $^{14}C$- or $^{13}C$-labeled phenylalanine, cinnamic acid, or glucose. In a classic study, Martin *et al.* (1980) followed the biodegradation and stabilization of specific C of model and cornstalk lignins, lignin monomer alcohols, and wheat straw over a 2-year incubation period using $^{14}C$-labeled substrates. The beta radiation emitted by $^{14}C$ makes it possible to follow small changes in C metabolism. The researchers labeled coniferyl alcohol with $^{14}C$ in specific C positions (A) and then fed it to corn plant cuttings to label the lignin fraction with $^{14}C$. (B) The corn and free coniferyl alcohol was then added to soil and incubated. In addition, $^{14}C$-labeled wheat straw labeled uniformly in a $^{14}CO_2$ environment was used for comparison. During the 2-year incubation under ideal moisture and temperature conditions, about 40% of the ring and two-side-chain C of coniferyl alcohol units were evolved as $CO_2$. Loss of $OCH_3$ carbons varied from 52 to 69%. The C mineralization rate was most rapid during 3 to 6 months of incubation. In comparison, total C losses from intact wheat straw and cornstalks after 2 years were about 72%. Most of the labeled lignin C was recovered from the humic acid fraction compared to the intact wheat straw. The $^{14}C$ of aromatics enters the microbial biomass to a very limited extent. However, an extrapolation of the decomposition data using an exponential function indicated that the same amount of lignin and intact plant residue C would remain in soil after 10 years of decomposition. Though it appears lignin is difficult to degrade, it does decompose at a rate fast enough not to accumulate in soil over short periods of time measured in decades.

BOX 12.3 (CONTINUED)




hydroxyl radicals (˙OH). This gives the brown rots the ability to degrade intact wood without completely disrupting the lignin structure. Representative organisms include *Poria* and *Gloeophyllum.*

White rot fungi are the most active lignin degraders. Several thousand species of white rots are known mainly from the basidiomycetes and ascomycetes. The basidiomycetes most studied are *Phanerochataete chrysosprium* and *Coriolus versicolor. Pleurotus ostreatus*, the oyster mushroom, and *Lintinula edodes*, the shiitake mushroom, are wood decay fungi that are grown commercially for food. Ascomycetes include *Xylaria*, *Libertella*, and *Hypoxylon.* They produce lignolytic

enzymes that oxidatively cleave phenylpropane units, demethylate, convert aldehyde groups (R–CHO) to carboxyl groups (R–COOH), and cleave aromatic rings, resulting in the complete destruction of lignin to $CO_2$ and water. Lignin degradation is repressed by more easily degraded substrates and very little lignin C is used for growth. White rots use three classes of extracellular lignin-degrading enzymes: the phenol oxidase laccase, lignin peroxidase, and manganese oxidase. Laccase and manganese peroxidase cannot directly oxidize nonphenolic structures, which constitute up to 70 to 90% of lignin. Lignin peroxidase can oxidize both phenolic and nonphenolic lignin structures. Working together, these enzymes can significantly degrade lignin but are not capable of penetrating the intact lignin structure because of their large molecular size. It is theorized that hydroxyl radicals, mentioned previously, enter the lignin structure and enlarge pores in the lignin structure to accommodate the movement of the larger enzymes (Watanabe, 2003). The $^{\bullet}OH$ radical may be produced from the reaction of Fe(II) with hydrogen peroxide ($H_2O_2$) via the Fenton reaction:

$$\mathrm{Fe(II)} + H_2O_2 \rightarrow \mathrm{Fe(III)} + OH^- + {}^{\bullet}OH$$

Other transition metals like Cu may also be used in this process. Some white rots produce these low-molecular-weight oxidants through lipid peroxidation. These potent free radicals are capable of significant lignin degradation in the absence of the larger lignin-degrading enzymes.

The degree of phenol decomposition in lignins can be described by the relative distribution of acidic and aldehydic phenolic units within the vanillyl and syringyl phenol families. As lignin is degraded, carboxylic acid units are formed from the lignin polymer during cleavage of phenylpropanoid $C\alpha$–$C\beta$ bonds. This leads to an increase in carboxylic acid-containing phenolic units with respect to phenolic units with an aldehyde side chain. The change in the acid-to-aldehyde ratio for vanillyl and syringyl units reflects the degree of lignin degradation. Kögel (1986), using the above ratio, showed that the degree of lignin decomposition increased with increasing soil depth. This approach provides for a quantitative measure of the degree of lignin phenol degradation in soil, but not of the absolute turnover of the original plant material.

Emerging molecular techniques are providing a better understanding of lignin decomposition. Extensive information on genomes containing lignin peroxidase now exists. Fungal mutants in whom N does not repress lignase activity are also available to study the mode of action and the ecology of these organisms. The white rots, such as *P. chrysosporium*, do not compete well with soil organisms and may be restricted to high-lignin substrates such as woody debris, indicating a complex ecology surrounding lignin degradation.

## OTHER PLANT CELL WALL CARBOHYDRATES AND PROTEINS

Intimately associated with the cell wall carbohydrate and lignin framework is a network of proteins thought to impart structural integrity to the plant cell wall.

These structural proteins are enriched with glycoproteins containing hydroxyproline, proline, and glycine. Another class of structural proteins called extensin, similar to collagen, consists of repeating serine–hydroxyproline and tyrosine–lysine–tyrosine sequences (Fig. 12.10). This structural protein plays an important architectural role cross-linking lignin to the carbohydrate network and may comprise up to 15% of the primary cell wall (Lodish *et al.*, 2000). Most of the hydroxyprolines are glycosylated with chains of three or four arabinose residues, and the serines are linked to galactose, making extensin about 65% carbohydrate. In addition to providing structural integrity to the plant cell wall, the unique amino acid and glycosation sequence may prevent microbial attack by presenting unrecognizable cleavage sites to proteolytic enzymes. Pathogens may gain access to the middle lamella through their ability to degrade extensin. The degradation of extensin may provide access for enzymes to further the attack of the polysaccharide and lignin framework of plant cell walls. Extensin most likely provides a source of N during decomposition. Another group of soil glycoproteins is found in the product identified as glomalin in soil. This fairly stable fungal product is produced by arbuscular mycorrhiza and is said to be involved with aggregation (Rillig, 2004).

## PLANT SECONDARY COMPOUNDS

Plants produce an array of organic compounds that are not associated with growth and development. These are often called secondary metabolites or plant secondary compounds and are divided into three major groups: terpenoids, alkaloids, and phenylpropanoids. Many play a significant role against herbivory and microbial infection, as attractants for pollinators and seed dispersers, and as allelopathic agents. These ecological functions affect primarily other organisms and have a profound effect on decomposition processes. The polyphenolic compounds range in molecular weight from 500 to 3000 daltons. They readily precipitate proteins through tannin reactions. Two major classes of tannins termed condensed and hydrolyzable tannins are found in higher plants (Fig. 12.11). Condensed tannins, also referred to as proanthocyanidins, are polymers of three-ring flavanols joined with C–C bonds. Hydrolyzable tannins are further grouped into gallotannins and ellagitannins that are composed of gallic acid or hexahydroxydiphenic acid esters, respectively, linked to a sugar moiety. The tanning of leather is an example in which a natural product is protected from microbial attack. The rate of decomposition can be directly related to the content of plant secondary compounds (Fig. 12.12). The ability to precipitate proteins has been hypothesized to be a competitive advantage for those organisms that can utilize the tannin–protein complexes (Kraus *et al.*, 2003).

## ROOTS AND ROOT EXUDATES

The allocation of photosynthate below ground can vary from 10 to 70% of total NPP (Finlay and Soderstrom, 1992). This represents a potentially significant C input to soils compared to aboveground plant litter inputs. Roots contain all the

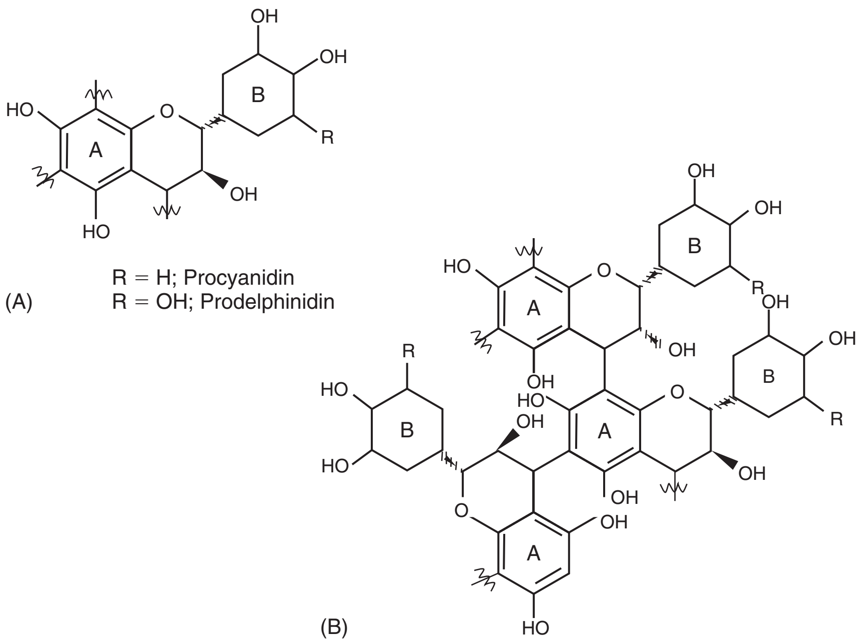



FIGURE 12.11 Structures of tannins. (A) A condensed tannin *trans* monomer unit and (B) a condensed tannin trimer showing different intermonomer linkages (C4–C8 and C4–C6).

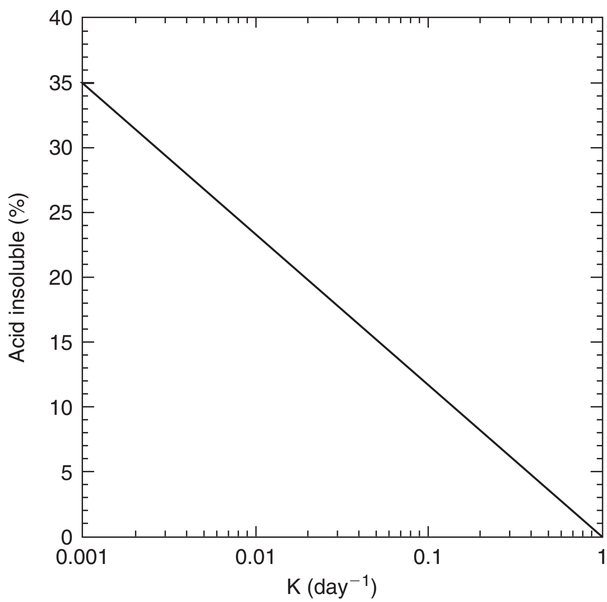



FIGURE 12.12 The influence of secondary compounds or acid-insoluble substances (%) on the rate of decomposition.

major structural components described above. Root exudates are the more easily decomposed simple carbohydrates (sugars) and amino compounds. Roots contribute mucilage from their growing tips, sloughed root cells, and exudates to soil. A significant portion of root exudates are nutrients, especially N (He *et al.*, 2006). The amount of C entering soil as root exudates has been fiercely debated mainly due to the complexity of studying roots *in situ.* Complicating this debate is the role of mycorrhizas in contributing C. The majority of C allocated below ground is respired within 2 weeks by the roots, mycorrhiza, and rhizosphere organisms. Root exudates are important substrates that contribute to rhizosphere processes, but their role in C cycling is not well understood. Natural grasslands labeled with $^{14}CO_2$ retained 52% of the assimilated C above ground and 36% in belowground structures (Milchunas *et al.*, 1985). In a field tree labeling experiment, only 20% of assimilated $^{14}CO_2$ was found in *Populus eugenii* roots 2 weeks following labeling regardless of season (Horwath *et al.*, 1994). The contribution of roots, although varying widely among different species, is often underestimated, leaving uncertainty in C budgets that aim to understand sources of C that maintain and sequester soil C.

## CELL WALLS OF MICROORGANISMS

Microbial cell walls have been shown to both accumulate in soil and become byproducts for microbial growth and synthesis of humic substances. Many different bacteria such as *Streptomyces*, *Pseudomonas*, *Bacillus*, and *Clostridium* degrade fungal cell walls. A major component of fungal cell walls is chitin, which contributes significant quantities of amino sugars to soil. The acetylglucosamine structure of chitin (Fig. 12.13) contains N, making its degradation unlikely to be limited by N. Recent studies have shown that available soil N is highly related to amino sugar content. Most amino sugars in soil are of microbial origin. The cell walls of *Phytophthera* contain fibers of cellulose and $\beta$(1-4) glucans that may interact with other cell components to affect its decomposition. Polymers of $\beta$(1-3) and $\beta$(1-6)

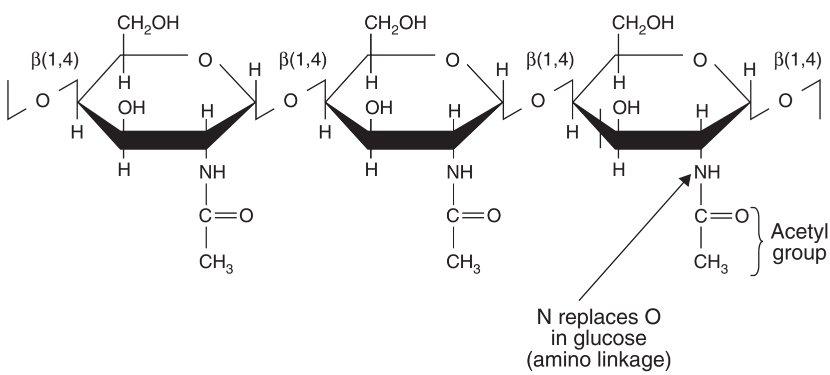



FIGURE 12.13 The structure of chitin showing the $\beta$(1-4) linking acetylglucosamine residues.

glucan are found in organisms such as *Fusarium.* Hyphae containing both chitin and glucans require both chitinase and glucanase to be degraded. Dark-colored pigments, often referred to as melanins, provide further protection and contribute to the low turnover rates of fungal cell wall constituents.

Bacterial cell walls are composed of a rigid layer of two sugar derivatives, *N*-acetylglucosamine and *N*-acetylmuramic acid chains. Gram-negative bacteria contain additional layers outside the ridged layer, creating more complex cell walls. These chains are linked by a limited number of amino acids through peptide bonds (Fig. 12.14). The cross-linkage peptidoglycan (murein) structures are composed of repeating units of L-alanine, D-alanine, D-glutamic acid, and either lysine or diaminopimelic acid. These components are connected to form a repeating structure called the glycan tetrapeptide. In $G^-$ bacteria, about 10% of the cell wall is peptidoglycan, in contrast to $G^+$ bacteria, in which peptidoglycan can make up to 90% of the cell wall. The thick wall of $G^+$ bacteria contains peptidoglycans linked to other wall constituents that include a variety of polysaccharide and polyphosphate

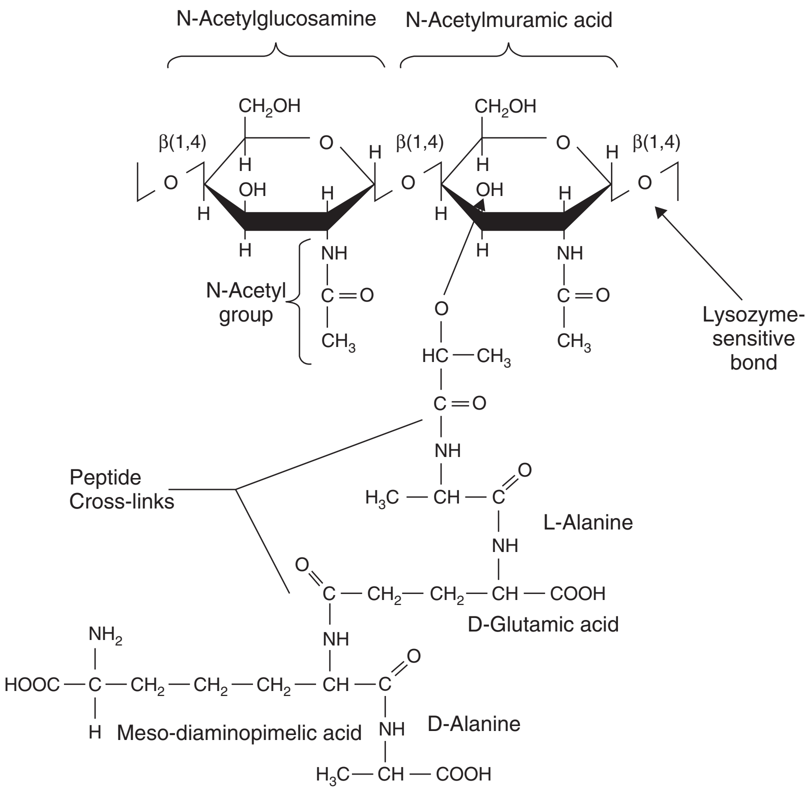



FIGURE 12.14 The structure of one of the repeating units of peptidoglycan found in gram-negative bacteria.

molecules joined through phosphoid–ester linkages. Most contain D-alanine. More than 100 different types of peptidoglycans have been identified.

## SOIL ORGANIC MATTER

The study of SOM began two centuries ago because of the recognition that it influences soil quality and plant growth. SOM contributes directly to plant and microbial growth through its effects on the chemical, physical, and biological properties of soil. Soil organic matter is the primary source of the essential nutrients N, P, and S. The association of SOM with secondary minerals such as clay and amorphous oxides to form organomineral complexes creates soil structure through the formation of aggregates. Aggregate formation enhances soil physical structure by organizing soil mineral grains and promoting aeration and water infiltration and storage. Good soil structure promotes plant growth and creates a labyrinth of habitats for microbial activity. The formation and decay of SOM is an essential process regulating atmospheric trace gases, particularly the production of $CO_2$ and nitrous oxide and consumption of methane. Another important consequence of the interactions of SOM with minerals is the creation of hydrophilic and hydrophobic domains that affect the adsorption of pesticides and other toxic compounds. Environmental toxicologists often refer to these domains as rubbery and glassy C, respectively, to explain the degree of adsorption and sorption of various chemical compounds in soil.

### SOIL ORGANIC MATTER FORMATION

The decomposition activities and life strategies of soil microorganisms and fauna act as the “waste management” crew of an ecosystem and play an important role in maintaining the global C budget by balancing the $CO_2$ fixed through photosynthesis and releasing $CO_2$ back to the atmosphere by decomposition. In temperate ecosystems, a small fraction of the C in NPP and NSP is preserved because it either becomes metabolized to a recalcitrant state, such as humic substances, or is protected physically through association with secondary minerals and soil aggregates. In boreal ecosystems, cold temperatures and water logging (peat soils) impede decomposer activity, leading to the accumulation of soil C as plant residues or particulate organic matter (POM). Permafrost areas such as tundra accumulate significant soil C and POM because low temperatures decrease microbial activity.

Soil organic matter consists of unrecognizable partially decayed plant residues, soil microorganisms, soil fauna, and the by-products of decomposition that lead to the production of humic substances. This process is called humification. The formation of humic substances results from many events of oxidation and hydrolysis that create materials with increased C and H and lower O content compared to the original animal, microbial, and plant tissue (Fig. 12.15). During the repeated degradation, N compounds react through free-radical coupling and increase the

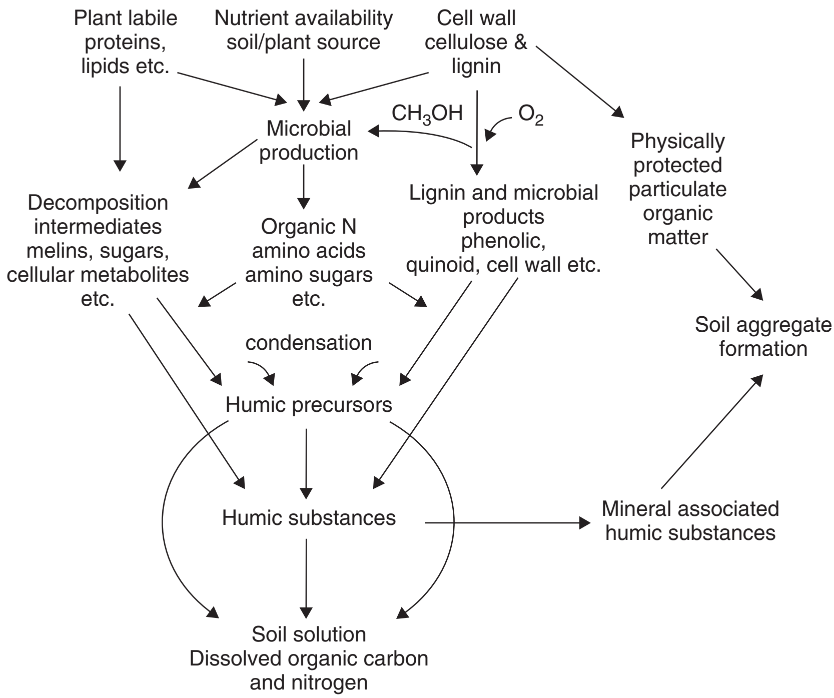



FIGURE 12.15 Hypothesized mechanism for the formation and protection of humic substances showing the importance of microbial products and altered lignin components in condensation reactions and formation of soil aggregates.

N content of humic substances. Humic substances consist of approximately 50–55% C, 5% H, 33% O, 4.5% N, 1% S, and 1% P (Table 12.3). Other metals and micronutrients, such as Al, Ca, Zn, and Cu, are present in much smaller amounts.

The humification process is analogous to lignin formation in that precursors are enzymatically produced or altered and the synthesis is characterized by condensation into larger molecules or structures. As with lignin, no two humic substances are alike in structure, but they behave similarly in function. The general chemical and structural properties of SOM are similar in soils found around the earth and appear to be a universal outcome of microbial decomposition processes in association with humification process and interactions with secondary minerals. As humans begin to explore the solar system the discovery of humic substances on distant planets may suggest past or present life. Since extraterrestrial simple organic compounds such as amino acids are found in meteorites it would be of interest to determine if prebiotic humic compounds also occur on asteroids.

There has been considerable debate about whether plant materials are directly incorporated into humic substances or are altered through microbial activity (Shevchenko and Bailey, 1996). Waksman (1936) theorized that lignin and proteins condensed to form humic acids, with the latter being of microbial origin. This has

**TABLE 12.3** Elemental and Functional and Constituent Group Components of Humic and Fulvic Acids[a]

| | Elemental analysis (%) | | | | | |
|---|---|---|---|---|---|---|
| Fraction | C | H | N | S | O | Ash |
| Fulvic acid | 49.5 | 4.5 | 0.8 | 0.3 | 44.9 | 2.4 |
| Humic acid | 56.4 | 5.5 | 4.1 | 1.1 | 32.9 | 0.9 |
| | Functional group analysis (meq $g^{-1}$) | | | | | |
| | $OCH_3$ | COOH | Phenolic OH | Total acidity | | |
| Fulvic acid | 05 | 9.1 | 3.3 | 12.4 | | |
| Humic acid | 10 | 4.5 | 2.1 | 6.6 | | |
| | Constituent groups (%) | | | | | |
| | Carboxyl C | Aromatic C | C–O; C–N–C | Aliphatic | | |
| Fulvic acid | 12.0–14.8 | 11.3–17.7 | 47.3–51.9 | 21.3–24.6 | | |
| Humic acid | 13.4 | 14.3 | 49.3 | 23.0 | | |

[a]Adapted from Haider (1992).

been termed the "Lignin Theory" of SOM formation (Stevenson, 1994). The shortcoming of the Lignin Theory is that it cannot account for the increased N content of SOM relative to that of the initial residues.

Maillard (Stevenson, 1994) suggested that the accumulation of N occurred through the interaction of reducing sugars and amino acids to form dark brown polymers similar to humic acids through a process termed the "Browning" reaction. The Browning reaction gained recognition because microorganisms readily produce sugar and amino compound substrates. In addition, the mechanisms could explain the formation of humic substances in aquatic environments, such as the ocean, where no lignin exists. The mechanism involves rearrangement and fragmentation of compounds to form three intermediates: (1) aldehydes and ketones, (2) reductones, and (3) furfurals. All of these react readily with amino compounds to form dark-colored end products through the formation of double bonds. Jokic *et al.* (2004) suggest that the action of manganese(IV) oxide on sugar–amino acid condensation under ambient conditions results in the formation of heterocyclic N compounds, suggesting that soil redox reactions may be sufficient to promote this reaction.

The polyphenol theory has recently become popular for describing the formation of SOM (Fig. 12.16). Polyphenols result from the decomposition of lignin or are microbially synthesized. Oxidation and demethylation of hydroxy phenols and aromatic acids result in the formation of quinines. Quinones polymerize into dark-colored humic substances. The presence of amino acid greatly enhances the polymerization and condensation process. These form aminoquinone intermediates

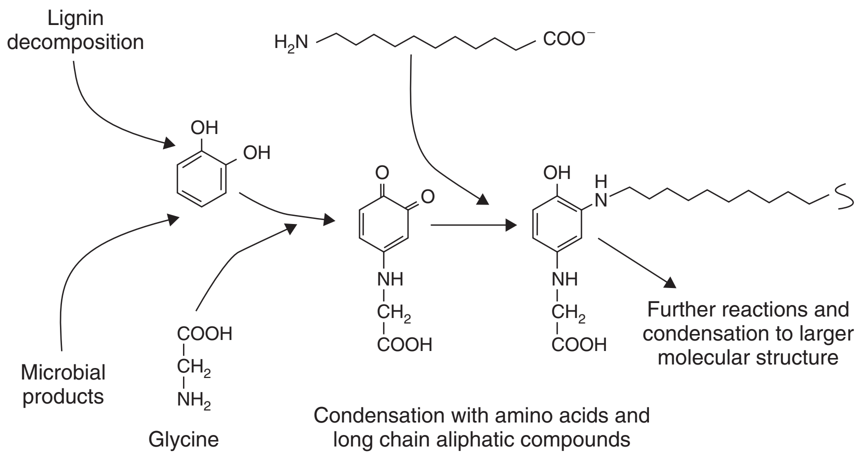



FIGURE 12.16 Hypothesized formation of a humic substance by reaction of a phenol decomposition product between an amino acid and an aliphatic substance.

upon oxidation to quinones and can condense to form brown, high-molecular-weight nitrogenous humates. The polyphenol reaction is considered important for forming SOM from lignin or melanin-degradation products (Haider *et al.*, 1975; Stevenson, 1994).

## CLASSICAL FRACTIONS OF SOIL ORGANIC MATTER

Chemical analyses have been used to probe the structure of SOM and define fractions that stabilize C and N and are important for controlling nutrient cycling. The classical extraction technique involves dispersion of soil in a strong base such as NaOH or $Na_4P_2O_7$ followed by acidification to fractionate different types of humic substances. The nonextractable fraction is termed humin. The dispersible fraction precipitated at low pH (<2) is known as humic acid. The dissolved fraction not affected by pH is termed fulvic acid. The extraction of humic substances depends on soil type or parent material. Soils formed from volcanic parent material tend to have greater humin fractions. Highly leached forested soils tend to have a higher proportion of fulvic acids. Grassland soils will usually have equal concentrations of all fractions. Much of the humin can be dispersed using a reductive agent such as sodium thiosulfate ($Na_2S_2O_3$) to counteract bridging cations such as Fe(III) to Fe(II) to remove mineral-associated humic substances. Pretreatment with HF to degrade the clay minerals will result in as much as 90–95% recovery, with the remaining C being referred to as black C (including charcoal) and acid-resistant waxy materials.

Fulvic acids are oxidized substances characterized by aromatic structures with extensive side chains and smaller N content compared to humic acids (Table 12.3). Fulvic acids of lower soil horizons, such as from a spodic B horizon, contain little N.

Fulvic acids of mollisols contain larger concentrations of N. Anoxic soil fulvic acids, such as those found in rice paddies, contain approximately 20 to 30% as much N as humic acids (Bird *et al.*, 2002). Humic acids are larger compounds of molecular weight 10,000 to 100,000 containing greater aromatic structure, cyclic N forms, and aliphatic and peptide residues. Humic acids contain 56% C, 5.5% H, 4% N, and 33% (Table 12.3). Compared to fulvic acids, humic acids contain more S and P. The functional groups are primarily COOH groups, phenolic OH groups, alcoholic groups, and a small amount of ketone oxygen. The high oxygen content of the fulvic acids relative to the humic acids is a result of much higher COOH (carboxyl) and phenolic OH content. Both fulvic and humic acids are adsorbed to clay minerals and hydrous oxides by polyvalent cations such as $Ca^{2+}$ and $Fe^{3+}$. The humin fraction is more strongly bound to minerals and has a C content in excess of 60%. It is enriched in fungal melanins and paraffinic substances compared to fulvic and humic substances.

## PHYSICAL ANALYSIS OF SOIL ORGANIC MATTER FRACTIONS

The physical fractionation of SOM by density and particle size yields fractions with distinctive chemical characteristics and turnover rates. Studies have shown that cultivation depletes soil of POM and mineral-associated fractions (Cambardella and Elliott, 1992; Tiessen and Stewart, 1983) immediately (Grandy and Robertson, 2006). Flotation in dense liquids such as cesium chloride, bromoform, sodium polytungstate, or silica gel with specific gravities ranging from 1.4 to 2.0 g $cm^{-3}$ yields the light fraction of SOM. The light fraction is considered to be POM characterized by it no longer being recognizable as plant residues, but not yet transformed into humic substances. The light fraction is both free in soil and protected in soil by entombment within aggregates. Separating soil particles by sieving or ultrasonification into specific aggregate size fractions followed by light fraction separation yields light fraction of varying turnover rates and capacity to influence nutrient cycling.

The light fraction and POM is 5 to 15% of the total C of cultivated soils and up to 25% or more in forest A horizons. The light fraction has been identified as a critical determinant of the cycling of nutrients and C. The depletion of light fraction reduces the capacity of soil to supply nutrients through mineralization processes. Particle size analysis of soil is useful to follow the transformation and stabilization of C and N from the addition of tracers or natural abundance isotopes such as $^{13}C$ from switches in plant photosynthetic types such as the $C_4 \leftrightarrow C_3$ species (Balesdent and Mariotti, 1987). Using these techniques, as well as $^{14}C$ enrichment studies and carbon dating, many studies have shown that about 20% of the total soil C turns over in less than 5 years, with the majority being light fraction and POM. The accumulation of POM and mineral-associated C in microaggregates residing within macroaggregates can be used as an early indication of long-term C sequestration as a result of changes in soil management such as tillage (Six *et al.*, 2002).

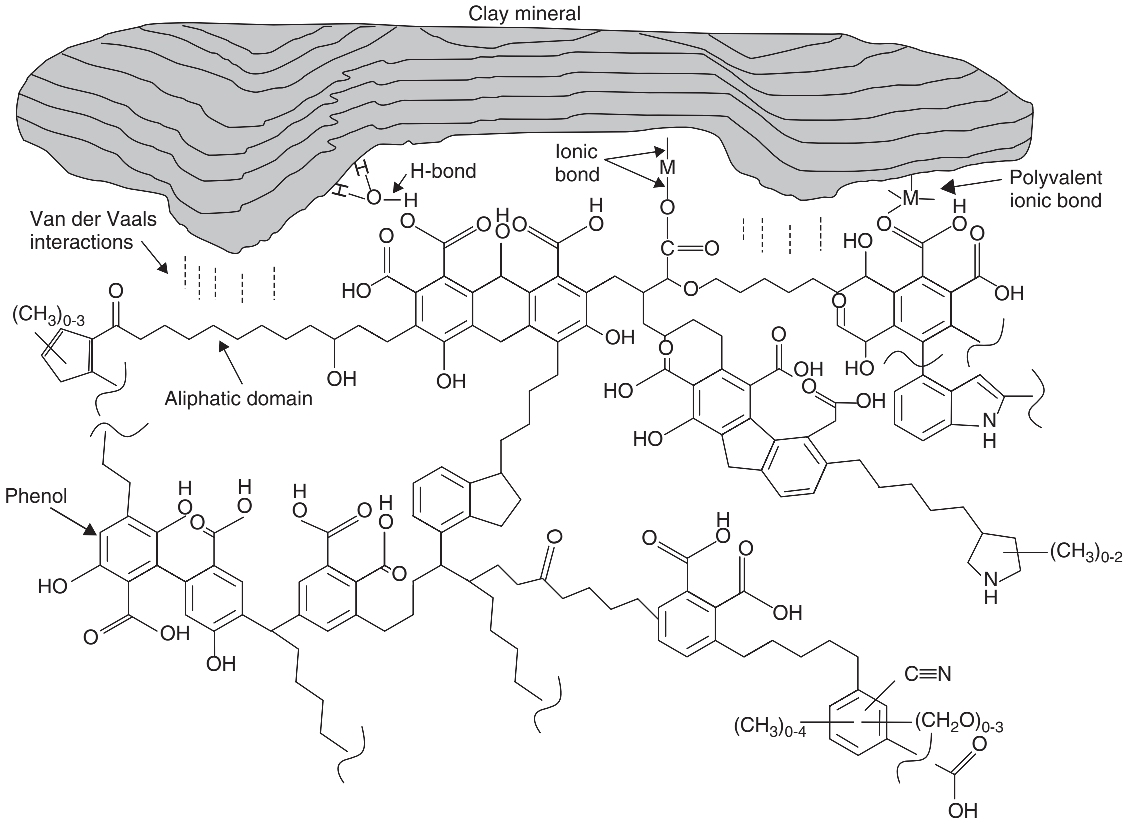



FIGURE 12.17 Idealized structure of humic acid showing high aliphatic content (adapted from Schulten and Schnitzer, 1993) showing physicochemical interactions with a clay mineral. Organomineral interactions M denotes various cations, such as iron and calcium.

## STRUCTURE OF SOIL ORGANIC MATTER

During the 1960s and 1970s, Schnitzer (1978) undertook exhaustive chemical oxidation and reduction to degrade humic substances into specific compounds. These procedures showed a significant aliphatic content of SOM (Fig. 12.17). The results also suggested that aromatic structures were cross-linked by longer chain aliphatic compounds. Schnitzer (1978) concluded that aliphatic compounds were more important structural features than had previously been assumed. Schulten *et al.* (1997), using pyrolysis-methylation gas chromatography/mass spectrometry, confirmed the results of chemical degradation studies. These studies found that the major structural components of SOM consisted of benzenes and *n*-alkyl benzenes. The alkyl–aryl compounds consisted of aromatic rings covalently bonded to aliphatic chains. They also showed that the aromatic fraction contained significant heterocyclic N (pyrroles and pyridines), N derivatives of benzene, and long-chain nitriles. Studies using $^{13}C$ NMR verified that SOM was not dominated by aromatic structures (Preston, 1996). $^{13}C$ NMR analysis has been useful for determining the chemical bond configurations of SOM that are often lost with extraction and chemical degradation methods. This research has provided insight into the chemical composition and structure of the SOM matrix and revealed possible mechanisms of humate interactions with clay minerals, metals, and anthropogenic compounds. However, this research fails to identify the active components of SOM that directly influence nutrient cycling processes.

# QUANTITY AND DISTRIBUTION OF ORGANIC MATTER IN SOILS

Soil organic matter plays a major role in sustaining ecosystems by regulating nutrient cycling and impacting physical properties as described earlier. The quantity of SOM is dependent on the balance between primary productivity and the rate of decomposition. The presence of silt and clay generally preserves some C from primary production and increases SOM under certain environments. The effects of climate, specifically moisture and temperature, primarily control the accumulation of soil C from its effect on both NPP and decomposition. The highest accumulation of C is found in swamps and marshes (723 t $ha^{-1}$) where NPP is high but decomposition is inhibited by a lack of $O_2$ (Table 12.4). Decomposition also is inhibited by cold in tundra soils and C tends to accumulate there primarily as litter and POM.

Humid tropical forests and boreal and temperate forests as well as temperate grasslands all accumulate approximately 200 t $ha^{-1}$ with turnover times of from 29 to 91 years as calculated by dividing SOM content by plant residue input. In contrast to temperate grassland, where C accumulates with an overall turnover time of 61 years, the low levels of SOM in tropical grassland have total turnover rates of only 10 years. As will be shown in the next chapter, SOM comprises a

TABLE 12.4 The Area, Stock of C, NPP, and C Turnover of Various Biomes[a]

| Biome | Area ($10^9$ ha) | Global C stock Plant | Global C stock Soil | Global C stock Total | NPP (Pg C/year) | Turnover (years)[b] |
|---|---|---|---|---|---|---|
| Tropical forests | 1.76 | 340 | 213 | 553 | 17.8 | 38 |
| Temperate forests | 1.04 | 139 | 153 | 292 | 7.3 | 29 |
| Boreal forests | 1.37 | 57 | 338 | 395 | 2.9 | 91 |
| Tropical savannas and grasslands | 2.51 | 79 | 247 | 326 | 16.3 | 10 |
| Temperate grasslands and shrublands | 1.52 | 23 | 176 | 199 | 6.15 | 61 |
| Deserts and semideserts | 3.66 | 10 | 159 | 169 | 2.45 | 37 |
| Tundra | 0.76 | 2 | 115 | 117 | 0.75 | 490 |
| Croplands | 1.48 | 4 | 165 | 169 | 5.45 | 21 |
| Wetlands | 0.35 | 15 | 225 | 240 | 4.3 | 520 |
| Total | 15.0 | 669 | 1791 | 2460 | 63.4 | |

[a]Adapted from Houghton *et al.* (2001).

[b]Adapted from Raich and Scheslinger (1992). Turnover of soil C assumes 30% of soil respiration is derived from root respiration.

number of fractions with different turnover rates, rather than the one value given for total SOM. The overall turnover time reflects the rapid turnover of the recent plant residues. The majority of the SOM turns over much more slowly, with mean residence times often of thousands of years for the humic fractions protected by clay mineral associations (Paul *et al.*, 2001).

## ROLE OF METHANE IN THE C CYCLE

Methane comprises less than 1% of the global C budget. Methane is found as natural gas in fossil fuel deposits, as hydrates or clathrates compounds in ice (e.g., permafrost), in the deep ocean, and in the atmosphere. Soil microorganisms both produce (methanogens) and consume (methanotrophs) $CH_4$. Microbial production of methane results from the decomposition of organic materials in the absence of oxygen. Carbon dioxide is used as an electron acceptor and a reduced organic compound is used as the donor. The reduction of $CO_2$ will occur in soil under extended reduced conditions such as in flooded environments or where oxygen diffusion is severely limited such as within soil aggregates. Waterlogged soils such as rice paddies, wetlands, waste disposal sites, and ruminant stomachs are typical examples of methanogenic habitats (Table 12.5). The production and distribution of fossil fuels contribute significantly to $CH_4$ emissions. Most of the annual flux of methane reacts with atmospheric hydroxyl radicals to form water and $CO_2$. Soil organisms consume about 10 to 30 Tg $CH_4$ year$^{-1}$, far below that required to mitigate emissions from anthropogenic sources but of the same magnitude as the annual atmospheric

TABLE 12.5 Global Sources and Sinks for Methane

| | Tg $CH_4$ $year^{-1}$ |
|---|---|
| Sources | |
| Wetlands | 86–115 |
| Fossil fuel production/distribution | 64–101 |
| Enteric fermentation/animal waste | 64–94 |
| Rice production | 44–60 |
| Biomass burning | 30–40 |
| Landfills | 30–49 |
| Termites | 20–153 |
| Oceans | 8–10 |
| Sinks | |
| Atmospheric removal | 308–560 |
| Soil microbial oxidation | 10–30 |
| Atmospheric increase | 28–32 |

With permission from Houghton *et al.* (2001).

loading rate. The loading rate is calculated as the difference between global production and consumption of $CH_4$, which results in an increase in atmospheric $CH_4$ of approximately 28 to 32 Tg $CH_4$ $year^{-1}$. Methane is a more potent greenhouse gas compared to $CO_2$ and its production and release has garnered much interest from the scientific community.

## FUTURE CONSIDERATIONS

The close link between NPP, NSP, and SOM and their influence on the sources and sinks for greenhouse gases indicate the importance of the global C cycle in regulating ecosystem productivity and climate. The major production and turnover of the components of the C cycle are fairly well characterized at the process level and microscale, for example in a gram of soil. However, on a global scale the interaction of organismal and metabolic diversity, sources and sinks for C, and anthropogenic influences have yet to be fully appreciated. These interactions must be better understood to adequately predict ecosystem response to perturbations such as climate change. Continued research on the biology and physical factors affecting the global C cycle is required to fully comprehend it.

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# 13

# Nitrogen Transformations

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P. M. Groffman



## INTRODUCTION

No other element essential for life takes as many forms in soil as nitrogen (N), and transformations among these forms are mostly mediated by microbes. Soil microbiology thus plays yet another crucial role in ecosystem function: in most terrestrial ecosystems N limits plant growth, and thus net primary production—the productive capacity of the ecosystem—can be regulated by the rates at which soil microbes transform N to plant-usable forms. However, several forms of N are also pollutants, so soil microbial transformations of N also affect human and environmental health, sometimes far away from the microbes that performed the transformation. Understanding N transformations and the soil microbes that perform them is thus essential for understanding and managing ecosystem health and productivity.

Nitrogen takes nine different forms in soil corresponding to different oxidative states (Table 13.1). Dinitrogen gas ($N_2$) is by far the most abundant form of N in the biosphere but is unusable by most organisms, including plants. Biological $N_2$ fixation, whereby $N_2$ is transformed to organic N (described in detail in Chap. 14), is the dominant process by which N first enters soil biological pools. All subsequent transformations are covered in this chapter: *N mineralization*, which is the conversion of organic N to inorganic forms; *N immobilization*, which is the uptake

TABLE 13.1 Main Forms of Nitrogen in Soil and Their Oxidation States

| Name | Chemical formula | Oxidation state |
|---|---|---|
| Nitrate | $NO_3^-$ | +5 |
| Nitrogen dioxide (g) | $NO_2$ | +4 |
| Nitrite | $NO_2^-$ | +3 |
| Nitric oxide (g) | NO | +2 |
| Nitrous oxide (g) | $N_2O$ | +1 |
| Dinitrogen (g) | $N_2$ | 0 |
| Ammonia (g) | $NH_3$ | −3 |
| Ammonium | $NH_4^+$ | −3 |
| Organic N | $R_{NH3}$ | −3 |

Gases (g) occur both free in the soil atmosphere and dissolved in soil water.

or assimilation of inorganic forms of N by microbes and other soil heterotrophs; *nitrification*, which is the conversion of ammonium ($NH_4^+$) to nitrite ($NO_2^-$) and then nitrate ($NO_3^-$); and *denitrification*, which is the conversion of nitrate to nitrous oxide ($N_2O$) and then dinitrogen gas ($N_2$). Other N species (Table 13.1) are involved in these conversions primarily as intermediaries, and during conversion can escape to the environment where they can participate in chemical reactions or be transported elsewhere for further reactions.

Löhnis (1913) first formulated the concept of the N cycle, which formalizes the notion that N species are converted from one form to another in an orderly and predictable fashion, and that at global equilibrium as much dinitrogen gas that is fixed each year by $N_2$ fixation must be annually converted back to $N_2$ gas via denitrification (Fig. 13.1).

The fact that $N_2$ fixation—both biological and industrial—now far outpaces historical rates of denitrification is the principal reason N has become a major modern pollutant (Galloway *et al.*, 2003). Making managed ecosystems more N conservative and removing N from waste streams are major environmental challenges that require a fundamental knowledge of soil microbial N transformations (Robertson, 1997).

Although the microbiology, physiology, and biochemistry of N cycle processes have been extensively studied for many decades, it is important to note that much of our understanding of these processes has been derived from molecular and organismal scale studies. In some cases, data from the laboratory have impaired our ability to understand and evaluate these processes in nature. Laboratory studies have characterized the nature and regulation of the processes that we discuss in this chapter, but the reductionist nature of these studies has caused us to overlook sometimes surprising possibilities for activity and regulating factors in the natural environment. The occurrence of denitrification (an anaerobic process) in dry and even desert soils is but one example: theory and years of laboratory work suggested that denitrification ought to occur only in wetland and muck soils, but when new field-based methods became available in the 1970s it became abundantly clear that almost all soils denitrify.

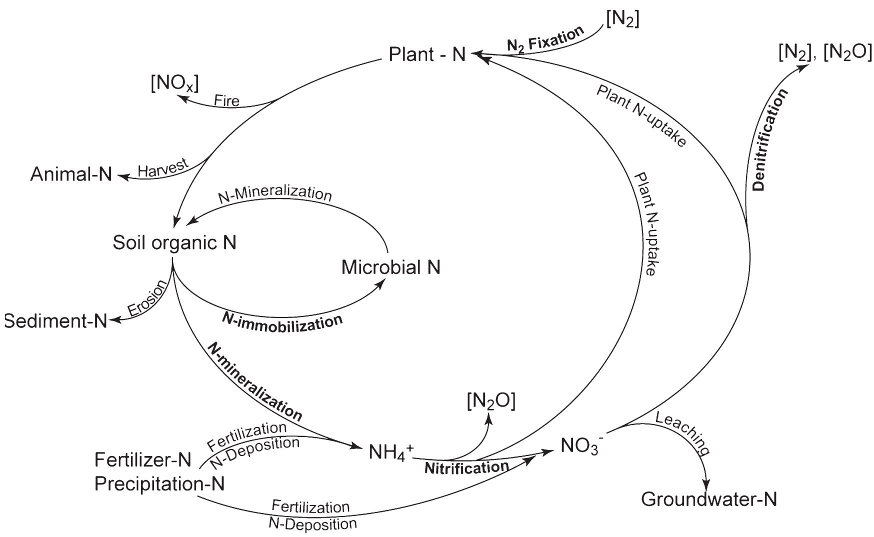



FIGURE 13.1 Schematic representation of the major elements of the terrestrial nitrogen cycle. Those processes mediated by soil microbes appear in bold. Gases appear in brackets.

Key problems have also arisen from evaluating microbial N cycle processes in isolation from other biogeochemical processes in nature (e.g., carbon (C) metabolism and plant nutrient uptake), from underestimating the physiological flexibility of bacteria in nature (e.g., nitrifying denitrifiers, aerobic denitrifiers, anammox bacteria), and from focusing almost exclusively on those microbes that can be cultivated in the laboratory. The disconnect between laboratory-derived knowledge and what actually occurs in the field is a problem throughout soil microbial ecology, but is perhaps most acute in the area of N cycling, which has great practical importance at field, landscape, regional, and global scales. It is when we attempt to scale up information from the microbial scale, to address real questions relating to plant growth, water pollution, and atmospheric chemistry at ecosystem, landscape, and regional scales, that this problem becomes obvious and important.

## NITROGEN MINERALIZATION AND IMMOBILIZATION

A critical process in any nutrient cycle is the conversion of organic forms of nutrients in dead biomass (detritus) into simpler, soluble forms that can be taken up again by plants and other microbes. This conversion is carried out by microorganisms that release, or mineralize, nutrients as a by-product of their consumption of detritus. While microorganisms attack detritus primarily as a source of energy and carbon to support their growth, they also have a need for nutrients, especially N, to assemble proteins, nucleic acids, and other cellular components. If plant

detritus is rich in N, microbial needs are easily met and N release, or mineralization, proceeds. If plant detritus is low in N, microorganisms must scavenge additional N from their surroundings, taking up or immobilizing N in their biomass.

The key to understanding mineralization–immobilization is to "think like a microbe" that is attempting to make a living by obtaining energy and C from detritus. Sometimes the detritus has all the N that the microbe needs, so as C is consumed, any extra N is released (mineralized) to the soil solution. Sometimes the detritus does not have enough N to meet microbial needs, so as C is consumed N is retained by the microbes and even more N must be immobilized from the soil solution. Indeed, it has been shown that microbes invest energy in the synthesis of enzymes (e.g., amidases to acquire N and phosphatases to acquire P) to acquire nutrients that they need while decomposing substrates of low quality. Microbial N uptake is also affected by organism growth efficiency. For example, fungi have wider C:N ratios in their tissues than bacteria and therefore—because of their lower N needs—can grow more efficiently on low-N substrates.

Mineralization results in an increase, while immobilization results in a decrease, in simple, plant-available forms of N in the soil. Traditionally, ammonium has been viewed as the immediate product of mineralization. In fact in the older literature mineralization is often referred to as ammonification. More recently, recognition of the fact that plants can take up simple, soluble organic forms of nutrients leads us to broaden our definition of mineralization products to include any simple, soluble forms of N that can be taken up by plants (see Schimel and Bennet, 2004). Plants from a variety of habitats have been shown to take up amino acids and other organic N forms; mycorrhizas play a role in this uptake by absorbing amino acids, amino sugars, peptides, proteins, and chitin that are then used by their hosts as an N source.

It is important to recognize that mineralization and immobilization are occurring at the same time within relatively small volumes of soil. While one group of microbes might be consuming a protein-rich and therefore nitrogen-rich piece of organic matter (think seed or leguminous leaf tissue), another group, perhaps 100 μm away, might be consuming detritus rich in C but low in N (think leaf stalk or wood). The first group is mineralizing N while the second is immobilizing it, perhaps even immobilizing the same N that is being mineralized by the first.

As a result of the simultaneous nature and small scale of these processes, it is also important to make a distinction between gross and net mineralization and immobilization. Gross N mineralization is the total amount of soluble N produced by microorganisms, and gross N immobilization is the total amount of soluble N consumed. Net N mineralization is the balance between the two. When gross mineralization exceeds gross immobilization, inorganic N in the soil is increasing, i.e., there is net mineralization. When gross immobilization exceeds gross mineralization, inorganic N in the soil is decreasing, i.e., there is net immobilization.

Mineralization and immobilization are carried out by a wide array of microorganisms—aerobes, anaerobes, fungi, and bacteria. Soil fauna also play an important role in mineralization and immobilization processes. They are responsible for much of the preliminary decomposition of detritus, they feed on and can regulate

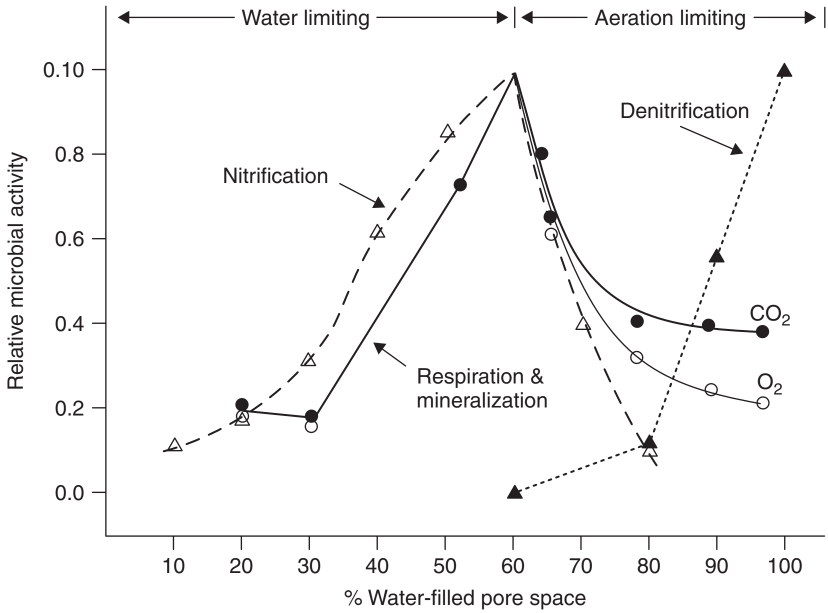



FIGURE 13.2 The relationship between water-filled pore space (a measure of soil moisture availability) and relative amount of microbial activities (redrawn from Linn and Doran, 1984).

populations of bacteria and fungi, and they can create or modify habitats for a wide array of organisms, e.g., earthworms create burrows and termites macerate wood. Mineralization and immobilization are widely distributed because they are so fundamental—all heterotrophic soil organisms consume organic materials for energy and C and immobilize and mineralize N as a by-product.

The widely distributed nature of mineralization and immobilization means that the environmental regulation of these processes is relatively straightforward. Rates of activity increase with temperature and are optimal at intermediate water contents, similar to respiration in Fig. 13.2, although it is important to recognize that significant activity is likely to occur at extremes of both temperature and moisture. In most soils the quantity and quality of detrital inputs are the main factors that control the rates and patterns of mineralization and immobilization. When moisture and temperature are favorable, large inputs of organic matter lead to high rates of microbial activity and the potential for high rates of mineralization and immobilization.

Water-filled pore space (WFPS) is a useful measure of moisture's influence on soil biological activity because it includes information about the impact of soil water on aeration in addition to information on water availability per se. The calculation of %WFPS is

$$\%\text{WFPS} = \frac{\text{soil water content} \times \text{bulk density} \times 100}{1 - (\text{bulk density}/2.65)}.$$

**TABLE 13.2** C:N Ratios in Various Organic Materials (from Tisdale *et al.*, 1993 and Hyvönen *et al.*, 1996)

| Organic material | C:N ratio |
|---|---|
| Soil microorganisms | 8:1 |
| Soil organic matter | 10:1 |
| Sewage sludge | 9:1 |
| Alfalfa residues | 16:1 |
| Farmyard manure | 20:1 |
| Corn stover | 60:1 |
| Grain straw | 80:1 |
| Oak litter | 200:1 |
| Pine litter | 300:1 |
| Crude oil | 400:1 |
| Conifer wood | 625:1 |

Soil water content is determined gravimetrically (g $H_2O$/g dry soil), bulk density (g $cm^{-3}$) is the oven dry weight of a given soil volume, and the value 2.65 is the density (g $cm^{-3}$) of rock—sand grains and other soil mineral particles.

What controls the balance between N mineralization and N immobilization? The answer is primarily organic matter quality—the availability of C in the material relative to its available N. Consider the effects of adding various materials with different C:N ratios to soil (Table 13.2). When one adds manure, with a relatively low C:N ratio (ca. 20:1), to soil, for example, the microbes have no trouble obtaining N and as a result mineralization dominates over immobilization, and plant-available N increases in soil. This is why manure is frequently used as a fertilizer. On the other hand, were one to add sawdust to soil, a material with a high C:N ratio (625:1), the microbes are keen to obtain the energy and C in the sawdust but cannot degrade this material without additional N because the sawdust does not have sufficient N to allow the microbes to build proteins. So the microbes must immobilize N from their environment, resulting in a decrease in plant-available N in the soil.

The balance between mineralization and immobilization is also affected by organism growth efficiency. For example, fungi have wider C:N ratios in their tissues than bacteria and, therefore, have a lower need for N and will thus mineralize N more readily. As a general rule of thumb, materials with a C:N ratio $>$25:1 stimulate immobilization, while those with a C:N ratio $<$25:1 stimulate mineralization (Table 13.2). The exception to this rule is highly decomposed substances with a low C:N ratio, e.g., soil organic matter (humus or compost) in which labile C and N have been depleted and the remaining C is in complex forms inherently resistant to decomposition (see Chap. 12) and therefore resistant to mineralization.

There are a wide variety of methods for measuring mineralization and immobilization (see Hart *et al.*, 1994; Robertson *et al.*, 1999). Measurement of net mineralization and immobilization rates is much easier and more common than is measurement of gross rates. Measurement of net rates usually involves quantifying

changes in inorganic N levels in some type of whole soil incubation. In most cases these incubations are in containers, with no plant uptake or leaching losses, and changes in inorganic N levels are measured by periodic extractions of the soil. Incubation methods vary widely, from short (10-day) incubations of intact soil cores buried in the field to long (>52-week) incubations of sieved soils in the laboratory. Gross rates are measured using isotope dilution methods whereby small amounts of $^{15}$N-labeled ammonium are added to the soil and the subsequent dilution of the label is used as a basis for calculating the gross production and consumption of ammonium.

## NITRIFICATION

Nitrification is the microbial oxidation of reduced forms of nitrogen to less reduced forms, principally $NO_2^-$ and $NO_3^-$. Autotrophic bacteria, first isolated in the late 1800s, gain as much as 440 kJ of energy per mole of $NH_3$ oxidized when $NO_3^-$ is the end product. We know now that heterotrophic microbes can also nitrify, although autotrophic nitrification appears to be the dominant process in most soils.

The importance of nitrifiers to ecosystem function is substantial: although some nitrate enters ecosystems in acid rain or as fertilizer, in most ecosystems nitrate is formed *in situ* via nitrification. Because nitrate is an anion, it is more mobile than ammonium, the ionized source of $NH_3$ in soil water:

$$NH_4^+(aq) \rightleftharpoons NH_3(aq) + H^+(aq).$$

As a positively charged ion, ammonium can be held on cation-exchange sites associated with soil organic matter, clay surfaces, and variable-charge minerals. Nitrate, on the other hand, can be easily transported out of the rooting zone by water when precipitation exceeds evapotranspiration.

Nitrate is also subject to denitrification, in which denitrifying bacteria convert nitrate to N gas. Additionally, nitrification in many soils is a major source of soil acidity, which can have multiple effects on ecosystem health, including the hydrologic loss of base cations as hydrogen ions displace other cations from exchange sites. And in soils dominated by variable charge minerals, which include most highly weathered tropical soils, soil acidity largely controls cation-exchange capacity (CEC), and nitrifier-generated acidity can drive CEC to nil. Further, some plants and microbes appear better able to take up ammonium than nitrate, and vice versa, implying a potential effect of nitrifiers on plant and microbial community composition. Finally, nitrifiers can also be direct sources of the atmospheric gases $NO_x$ and $N_2O$.

### THE BIOCHEMISTRY OF AUTOTROPHIC NITRIFICATION

Autotrophic nitrification is a two-step process, carried out by separate groups of bacteria: the ammonia and nitrite oxidizers, respectively. Autotrophic nitrifiers

derive their C from $CO_2$ or carbonates, rather than from organic matter, and are obligate aerobes. $NH_3$ oxidation is characterized as

$$NH_3 + 1\frac{1}{2}O_2 \rightarrow NO_2^- + H^+ + H_2O.$$

The first step in this oxidation is mediated by the membrane-bound enzyme ammonia mono-oxygenase, which can also oxidize a wide variety of organic, non-polar low-molecular-weight compounds, including phenol, methanol, methane, and halogenated aliphatic compounds such as trichloroethylene:

$$NH_3^+ + 2H^+ + O_2 + 2e^- \xrightarrow{\text{ammonia mono-oxygenase}} NH_2OH + H_2O.$$

The reaction is irreversibly inhibited by small quantities of acetylene, which inhibits ammonia mono-oxygenase and thereby provides a means for experimentally differentiating autotrophic from heterotrophic nitrification in soil. Hydroxylamine is further oxidized to nitrite by the reaction

$$NH_2OH + H_2O \xrightarrow{NH_2OH\text{ oxidoreductase}} NO_2^- + 4e^- + 5H^+.$$

Two of the four electrons released in this reaction are used in the prior $NH_3$ oxidation step; the remaining two are used in electron transport, generating energy for cell growth and metabolism:

$$2H^+ + \frac{1}{2}O_2 + 2e^- \xrightarrow{\text{terminal oxidase}} H_2O.$$

Intermediary compounds formed during the oxidation of hydroxylamine to nitrite can result in the formation of NO (Fig. 13.3), which can escape to the atmosphere and influence the photochemical production of ozone ($O_3$) and the abundance of hydroxyl (OH) radicals in air, primary oxidants for a number of tropospheric trace gases including methane. Ammonia oxidizers also appear able to produce NO via $NO_2^-$ reduction, which results in the production of $N_2O$, an important greenhouse gas that can also escape to the atmosphere. Nitrite reduction occurs when ammonia oxidizers use $NO_2^-$ as an electron acceptor when $O_2$ is limiting—effectively becoming denitrifying nitrifiers! Denitrification is described later in this chapter.

In most soils the nitrite produced by ammonia oxidizers does not accumulate but is quickly oxidized to nitrate by the nitrite-oxidizing bacteria when they perform nitrite oxidation:

$$NO_2^- + H_2O \xrightarrow{\text{nitrite oxidoreductase}} NO_3^- + 2H^+ + 2e^-.$$

These reactions are membrane-associated and because nitrite oxidoreductase is a reversible enzyme, the reaction can be reversed to result in nitrate reduction to nitrite. Up to 80% of the energy produced during nitrification is respired via the Calvin cycle; growth efficiencies of the nitrifiers are correspondingly low. This

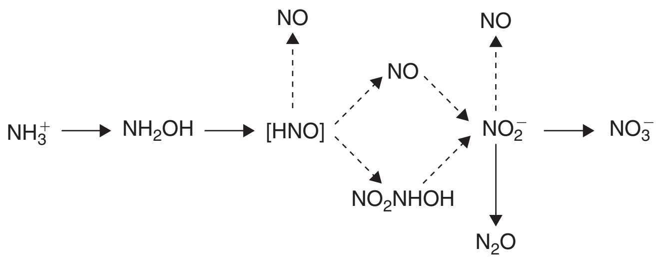



FIGURE 13.3 Autotrophic nitrification pathways including pathways for gas loss. Broken lines indicate unconfirmed pathways (from Firestone and Davidson, 1989).

explains in part their poor ability in many soils to compete with heterotrophs and plants for ammonium.

## THE DIVERSITY OF AUTOTROPHIC NITRIFIERS

For a group of microbes with such functional importance at a variety of scales, the nitrifiers are a remarkably depauperate group from a taxonomic standpoint. Although slow growth rates hinder cultivation-based analyses of their diversity, as do culture techniques that fail to reproduce the diversity of microhabitats in soil (see Chap. 3), even molecular methods fail to find much nitrifier diversity. There is, nevertheless, even in the single phylogenetic group to which most ammonia-oxidizing bacteria belong, significant sequence and physiological diversity. Moreover, the recent discovery of widespread ammonia-oxidizing Archaea bacteria in marine ecosystems (Francis *et al.*, 2005) suggests the potential for similar discoveries of new diversity in soil. Leininger *et al.* (2006) suggest that Archaea may be more numerous than bacterial ammonia oxidizers in soil.

From a taxonomic standpoint, bacterial nitrifiers are viewed as the single Family Nitrobacteraceae, defined by their characteristic ability to oxidize ammonia or nitrite, although biochemical and molecular evidence provides no justification for this view. Early work beginning with Winogradsky (1892) classified the ammonium-oxidizing genera of Nitrobacteraceae on the basis of cell shape and the arrangement of intracytoplasmic membranes. This yielded five genera: *Nitrosomonas*, *Nitrosococcus*, *Nitrosospira*, *Nitrosolobus*, and *Nitrosovibrio*. More recent work based principally on 16S rRNA oligonucleotide and gene sequence analysis places all of these groups in the beta subclass of the Proteobacteria but for a single species of *Nitrosococcus*, which is placed in the Gammaproteobacteria (Purkhold *et al.*, 2000; Fig. 13.4). Today we have almost complete 16S rRNA gene sequences for the 14 species of Betaproteobacteria ammonia oxidizers, which have a gene sequence similarity of 89% (Fig. 13.5; Koops *et al.*, 2003).

Of the ammonia oxidizers thus far isolated from soil all are in the Betaproteobacteria. In arable soils the *Nitrosomonas communis* lineage with strains of *Nitrosolobus multiformis* are numerically dominant. Unfertilized soils usually also contain strains of the *Nitrosomonas oligotropha* lineage and strains of *Nitrosospira*

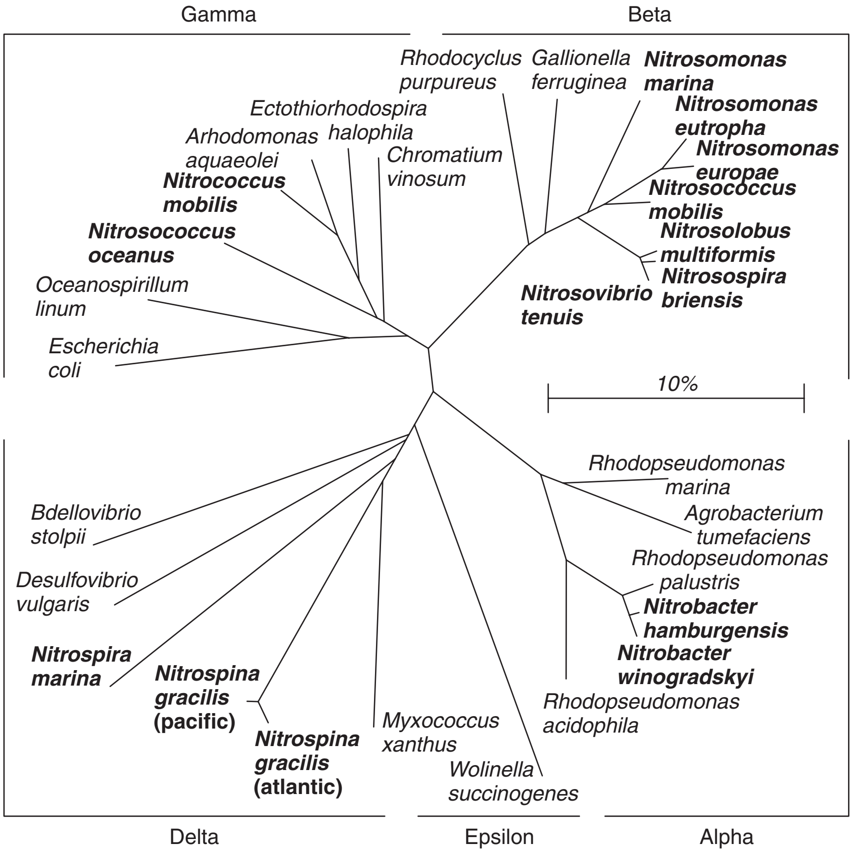



FIGURE 13.4 Distance tree for the Proteobacteria including nitrifiers (in bold). The scale bar corresponds to 0.1 estimated fixed mutation per sequence position (from Teske *et al.*, 1994). More recent work places *Nitrococcus mobilis* in the Betaproteobacteria (see Fig. 13.5).

and *Nitrosovibrio* (Koops and Pommerening-Röser, 2001). The latter two tend to be dominant in acid soils, which contain few if any *Nitrosomonas*.

Molecular techniques such as 16S rRNA sequences and the retrieval of *amoA* clones have also been used to examine the diversity of ammonia oxidizers *in vivo*, which avoids the need for pure-culture cultivation and its bias toward those species that are cultivatable outside of their native habitat. Although molecular techniques can themselves be biased because of their dependency on the extraction of nucleic acid from soil, PCR amplification, primer bias, and cloning methods, they mostly corroborate pure-culture findings: most *amoA* clones and 16S rRNA based surveys are similar to sequence clusters defined by cultured ammonia oxidizers. Nevertheless, few sequences are completely identical to those of cultured organisms, and since it is not possible to obtain DNA–DNA hybridization data for noncultured organisms, there is no way currently to know if these differences are sufficient to define different species.

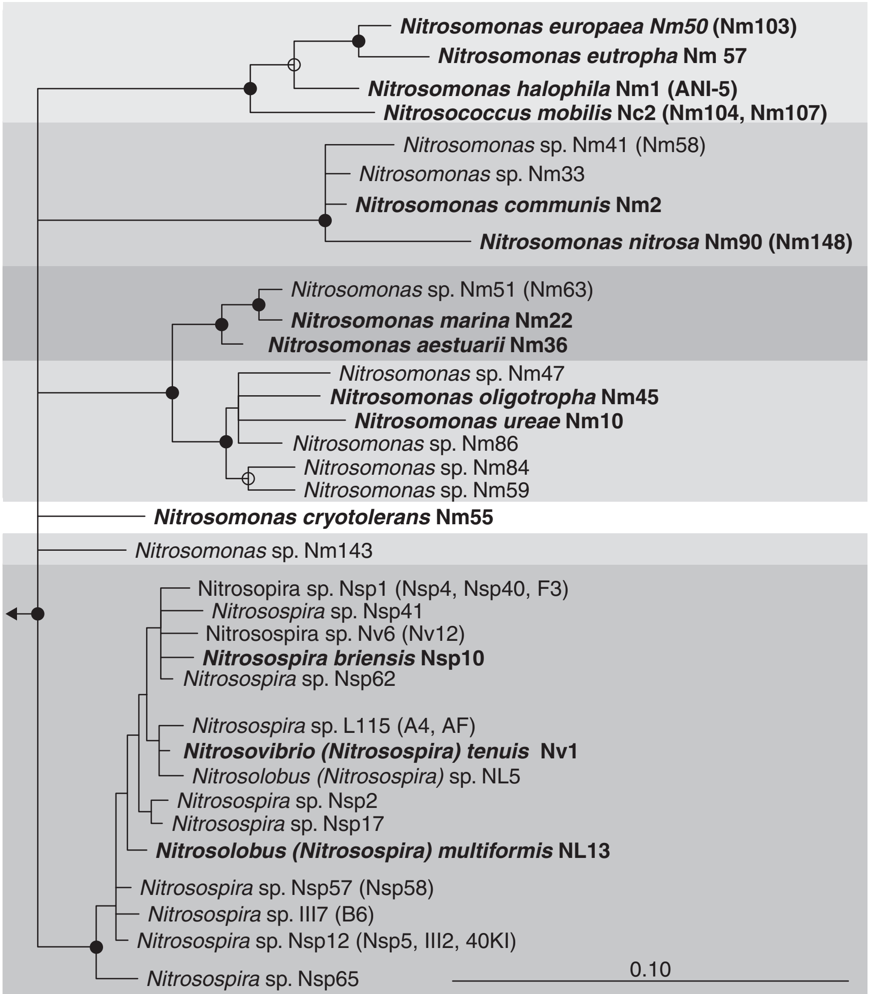



FIGURE 13.5 16S rRNA-based phylogenetic tree of the betaproteobacterial ammonia oxidizers. The tree includes only those oxidizers that have been demonstrated to represent different genospecies (DNA–DNA similarity <60%) and for which 16S rRNA gene sequences longer than 1000 nucleotides are available. Strains with DNA–DNA similarity >60% are in parentheses after the respective species name. Described species are depicted in bold. Scale bar represents 10% estimated sequence divergence (from Koops *et al.*, 2003).

Worth noting too is the fact that these techniques do not normally provide quantitative information about the abundance and activity of different species *in situ*. Quantitative PCR and newer techniques based on membrane or *in situ* hybridization in concert with rRNA-targeted probes (e.g., fluorescence *in situ* hybridization or FISH, as used in aquatic and wastewater treatment studies; Juretschko *et al.*, 1998) can directly relate community structure with activity and the spatial distribution of

targeted organisms. Recent work (e.g., Prosser and Embley, 2002) has shown how these techniques can be used to discover nitrifier community change in response to changes in ecosystem management and land use.

Nitrite-oxidizing bacteria appear in a broader array of phylogenetic groupings than do the ammonia oxidizers, but only the genera *Nitrobacter* and *Nitrospira* (Freitag *et al.*, 2005) have been detected in soil; the distribution of the other nitrite-oxidizing genera (*Nitrosococcus* and *Nitrospina*) is not fully known. Members of *Nitrobacter* form an exclusive and highly related cluster in the Alphaproteobacteria (Fig. 13.4). Pairwise evolutionary distance estimates are less than 1%, indicating little genetic diversity within the group (Fig. 13.5), a finding supported by 16S rRNA sequence comparisons (Teske *et al.*, 1994). The other nitrite-oxidizing genera are in the delta (*Nitrospina and Nitrospira*) and gamma (*Nitrosococcus*) subclasses of the Proteobacteria.

## HETEROTROPHIC NITRIFICATION

A wide variety of heterotrophic bacteria and fungi have the capacity to oxidize $NH_4^+$. So-called heterotrophic nitrification is not linked to cellular growth, as it is for autotrophic nitrification. There is evidence for two pathways for heterotrophic ammonia oxidation. The first is similar to that of autotrophic oxidation, in that the nitrifying bacteria have similar ammonia- and hydroxylamine-oxidizing enzymes. In fact these enzymes can oxidize a number of different substrates, and it may be that ammonia oxidation is only secondary to these enzymes' main purpose of oxidizing propene, benzene, cyclohexane, phenol, methanol, or any of a number of other nonpolar organic compounds.

The second heterotrophic pathway is organic and appears limited to fungi. It involves the oxidation of amines or amides to a substituted hydroxylamine followed by oxidation to a nitroso and then a nitro compound with the following oxidation states:

$$\begin{array}{ccccccccc} RNH_2 & \rightarrow & RNHOH & \rightarrow & RNO & \rightarrow & RNO_3 & \rightarrow & NO_3^- . \\ -3 & & -1 & & +1 & & +3 & & +5 \end{array}$$

These reactions are not coupled to ATP and thus produce no energy. Alternatively, N compounds may react with hydroxyl radicals produced in the presence of hydrogen peroxide and superoxide, which may happen when fungi release oxidases and peroxidases during cell lysis and lignin degradation.

Heterotrophic bacteria such as *Arthrobacter globiformis, Aerobacter aerogenes, Thiosphaera pantotropha*, *Streptomyces grisens*, and various *Pseudomonas* spp. have been found to nitrify. The fungi *Aspergillus flavus* was first isolated as a nitrifier in 1954 and is the most widely studied of the nitrifying heterotrophs.

Interest in heterotrophic nitrification increased substantially in the late 1980s when it became clear that accelerated inputs of atmospheric ammonium to acid forest soils were being nitrified to nitrate with alarming effects on soil acidity, forest health, and downstream drinking water quality. It was assumed until recently

that most of this nitrification was heterotrophic; we know now that most nitrification in acid soils is autotrophic (De Boer and Kowalchuk, 2001), although the exact mechanisms by which nitrification occurs at low pH are not well understood. Heterotrophic nitrification thus appears important in some soils and microenvironments, perhaps particularly where autotrophic nitrifiers are chemically inhibited (see below), but are thought now to rarely dominate the soil nitrifier community.

## ENVIRONMENTAL CONTROLS OF NITRIFICATION

The single most important factor regulating nitrification in the majority of soils is ammonium supply (Fig. 13.6). Where decomposition and thus N mineralization is low or where $NH_4^+$ uptake and thus N-immobilization by heterotrophs or plants is high, nitrification rates will be low. Conversely, any ecosystem disturbance that increases soil $NH_4^+$ availability will usually accelerate nitrification unless some other factor is limiting. Tillage, fire, clear-cutting, waste disposal, fertilization, atmospheric N deposition—all have well-documented effects on nitrate production in soils, mostly due to their effects on soil $NH_4^+$ pools.

That nitrification usually accelerates only when the $NH_4^+$ supply exceeds plant and heterotroph demand implies that nitrifiers are relatively poor competitors for $NH_4^+$ in the soil solution. In fact this is the case: nitrification rates are typically low in midsuccessional communities and aggrading forests because of high plant demand for N, and also following the addition of high C:N residues to agricultural soils because of high microbial (heterotroph) demand for N. In old-growth forests and mature grasslands, plant N demand has diminished and consequently nitrification is usually higher than in midsuccessional communities in which plant biomass is still accumulating, but not usually as high as in early successional communities, in which N supply often greatly exceeds demand (Robertson and Vitousek, 1981).

Oxygen is another important regulator of nitrification in soil. All known nitrifiers are obligate aerobes, and nitrification proceeds very slowly if at all in submerged soils. In flooded environments such as wetlands and lowland rice, nitrifiers are active only in the oxidized zone around plant roots and at the water–sediment interface, usually only a few millimeters thick. And although some nitrifiers have the capacity to use nitrite rather than $O_2$ as an electron acceptor during respiration, $O_2$ is still required for ammonia oxidation.

Nitrifiers are little different from other aerobic microbes with respect to their response to temperature, moisture, and other environmental variables (see Fig. 13.2). Nitrification occurs slowly but readily under snow and in refrigerated soils, and soil transplant experiments (e.g., Mahendrappa *et al.*, 1966) have demonstrated an apparent capacity for nitrifiers to adapt to different temperature and moisture regimes. For many decades nitrifiers were thought to be inhibited in acid soils, probably because in many cases and especially in soils from cultivated fields, raising soil pH with calcium or magnesium carbonate stimulates nitrification, and culturable nitrifiers exhibit a pH optimum of 7.5–8 (Prosser, 1989). We now recognize that nitrification can be high even in very acid forest soils ($pH < 4.5$;

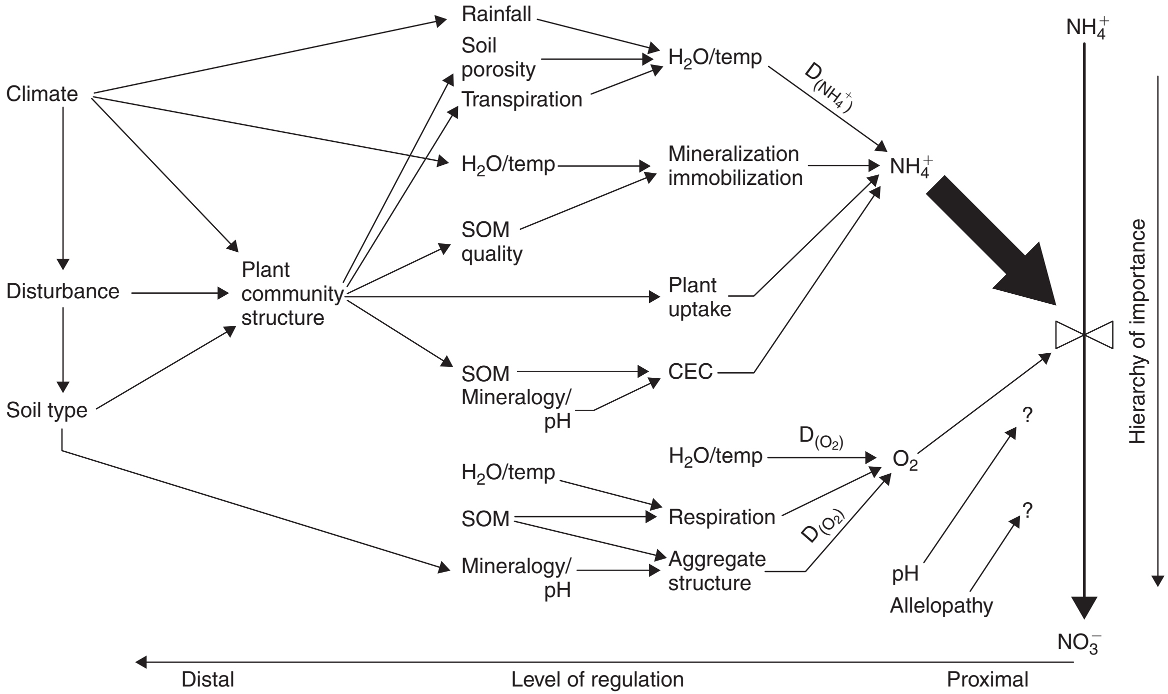



FIGURE 13.6 Environmental controls on nitrification (from Robertson, 1989, after Groffman *et al.*, 1988). The most proximal scale (right side) is at the cellular level.

Robertson, 1989), although the physiological basis for this is still not well understood (DeBoer and Kowalchuck, 2001).

## INHIBITION OF NITRIFICATION

Nitrification is unaccountably slow in some soils, and in some circumstances it may be inhibited by natural or manufactured compounds. A wide variety of plant extracts can inhibit culturable nitrifiers *in vitro*, for example, although their importance *in situ* is questionable. Likewise, commercial products such as nitrapyrin and dicyandimide can be used to inhibit nitrification in soil with varying degrees of success. Most commercial compounds are pyridines, pyrimidines, amino triazoles, and sulfur compounds such as ammonium thiosulfate. One recent innovation is paraffin-coated calcium carbide ($CaC_2$; Freney *et al.*, 2000). Calcium carbide reacts with water to form acetylene ($C_2H_2$), which inhibits nitrifiers at very low partial pressures, ca. 10 Pa. As the paraffin wears off, $CaC_2$ is exposed to soil moisture and the $C_2H_2$ formed inhibits nitrification. Likewise, neem oil, extracted from the Indian neem tree (*Azadirachta indica*), has been used commercially to coat urea fertilizer pellets to slow its nitrification to $NO_3^-$.

The potential value of managing nitrifiers in ecosystems can be easily seen from the position of nitrification in the overall N cycle (Fig. 13.1). Nitrogen is lost from ecosystems mainly after its conversion to $NO_3^-$ and prior to plant uptake, so keeping N in the $NH_4^+$ form keeps it from being lost by nitrate leaching and denitrification, the two principal pathways of unintentional N loss in most ecosystems. Because many plants prefer to take up N as $NO_3^-$, it is not desirable to inhibit nitrification completely even in intensively managed ecosystems such as fertilized row crops, but slowing nitrifiers or restricting their activity to periods of active plant growth is an attractive—if still elusive—management option.

## DENITRIFICATION

Denitrification is the reduction of soil nitrate to the N gases NO, $N_2O$, and $N_2$. A wide variety of mostly heterotrophic bacteria can denitrify, whereby they use $NO_3^-$ rather than oxygen ($O_2$) as a terminal electron acceptor during respiration. Because nitrate is a less efficient electron acceptor than $O_2$, most denitrifiers undertake denitrification only when $O_2$ is otherwise unavailable. In most soils this occurs mainly following rainfall as soil pores become water-saturated and the diffusion of $O_2$ to microsites is slowed drastically. Typically denitrification starts to occur at water-filled pore space concentrations of 60% and higher (Fig. 13.2). In wetland and lowland rice soils diffusion may be restricted most of the time. Oxygen demand can also exceed supply inside soil aggregates and in rapidly decomposing litter.

Denitrification is the only point in the N cycle at which fixed N reenters the atmosphere as $N_2$; it thus serves to close the global N cycle. In the absence of denitrification, $N_2$ fixers (see Chap. 14) would eventually draw atmospheric $N_2$ to nil,

and the biosphere would be awash in nitrate. Denitrification is also important as the major source of atmospheric $N_2O$, an important greenhouse gas that also consumes stratospheric ozone.

From a management perspective, denitrification is advantageous when it is desirable to remove excess $NO_3^-$ from soil prior to its movement to ground or surface waters. Sewage treatment often aims to remove N from waste streams by managing nitrification and denitrification. Typically wastewater is directed through sedimentation tanks, filters, and sand beds designed to remove particulates and encourage decomposition and the mineralization of organic N to $NH_4^+$, which is then nitrified under aerobic conditions to $NO_3^-$. The stream is then directed to anaerobic tanks where denitrifiers convert the $NO_3^-$ to $N_2O$ and $N_2$, which is then released to the atmosphere. Part of the nitrification/denitrification management challenge is ensuring that the stream is exposed to aerobic conditions long enough to allow nitrifiers to convert most $NH_4^+$ to $NO_3^-$ but not so long as to remove all dissolved organic C (known as biological oxygen demand or BOD to wastewater engineers), which the denitrifiers need for substrate.

Denitrification can also remove nitrate from groundwater prior to its movement to streams and rivers. In most wetlands and riparian areas nitrate-rich groundwater must move across a groundwater–sediment interface that is typically anaerobic and carbon-rich. As nitrate moves across this interface it can be denitrified to $N_2O$ and $N_2$, keeping it from polluting downstream surface waters.

In managed ecosystems it is usually desirable to minimize denitrification in order to conserve N further for plant uptake; in regions with ample rainfall ecosystem N losses due to denitrification can rival or exceed losses by nitrate leaching. There are no technologies designed to inhibit denitrification per se; usually denitrifiers are best managed indirectly by manipulating water levels (e.g., in rice cultivation) or nitrate supply (e.g., nitrification inhibitors).

## DENITRIFIER DIVERSITY

Denitrification is carried out by a broad array of soil bacteria, including organotrophs, chemo- and photolithotrophs, $N_2$ fixers, thermophiles, halophiles, and various pathogens. Over 50 genera with over 125 denitrifying species have been identified (Zumft, 1992). In soil, most culturable denitrifiers are facultative anaerobes from only 3–6 genera, principally *Pseudomonas* and *Alcaligenes* and to a lesser extent *Bacillus*, *Agribacterium*, and *Flavibacterium.* Typically denitrifiers constitute 0.1 to 5% of the total culturable soil population and up to 20% of total microbial biomass (Tiedje, 1988).

Organisms denitrify to generate energy (ATP) by electron transport phosphorylation via the cytochrome system. The general pathway is

$$2NO_3^- \xrightarrow[nar]{} 2NO_2^- \xrightarrow[nir]{} 2NO^{\uparrow} \xrightarrow[nor]{} N_2O^{\uparrow} \xrightarrow[nos]{} N_2^{\uparrow}.$$

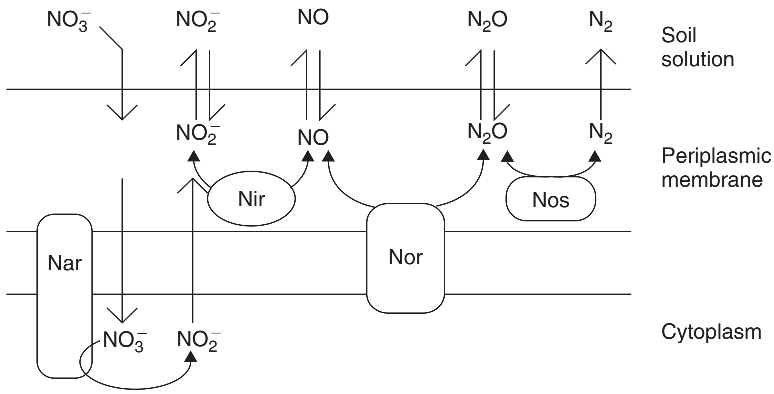



FIGURE 13.7 The organization of denitrification enzymes in the cell membrane for gram-negative bacteria (adapted from Ye *et al.,* 1994).

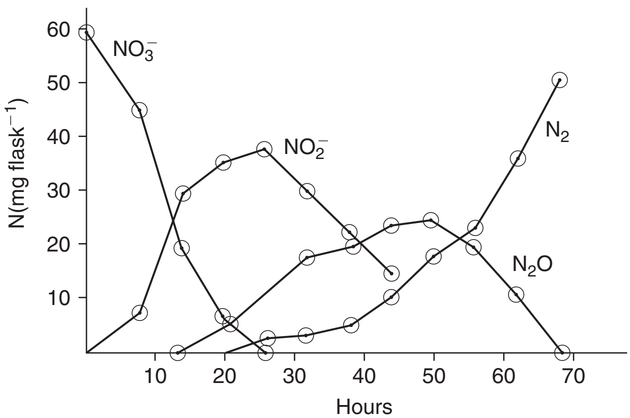



FIGURE 13.8 The sequence of products formed during denitrification (adapted from Cooper and Smith, 1963).

Each step is enacted by individual enzymes: nitrate reductase (*nar*), nitrite reductase (*nir*), nitric oxide reductase (*nor*), and nitrous oxide reductase (*nos*). Each is inhibited by $O_2$, and the organization of these enzymes in the cell membrane for gram-negative bacteria is described in Fig. 13.7. At any step in this process intermediate products can be exchanged with the soil environment, making denitrifiers a significant source of $NO_2^-$ in soil solution and important sources of the atmospheric gases NO and $N_2O$.

Each denitrification enzyme is inducible, primarily in response to the partial pressure of $O_2$ and substrate (C) availability. Because enzyme induction is sequential and substrate dependent, there is usually a lag between the production of an intermediate substrate and its consumption by the next enzyme. In pure culture, these lags can be on the order of hours (Fig. 13.8); in the field lags can be substantially

longer, and differences in lags among different microbial taxa may significantly affect the contribution of denitrifiers to fluxes of NO and $N_2O$ to the atmosphere. That induced enzymes degrade at different rates, and more slowly than they are induced, also leads to a complex response to the environmental conditions that induce denitrification; whether a soil has denitrified recently (whether denitrifying enzymes are present) may largely determine its response to newly favorable conditions for denitrification. Rainfall onto soil that is moist, for example, will likely lead to a faster and perhaps stronger denitrification response than will rainfall onto the same soil when it is dry (Groffman and Tiedje, 1988; Bergsma *et al*. 2002).

## ENVIRONMENTAL CONTROLS OF DENITRIFICATION

For decades after its discovery as an important microbial process, denitrification was assumed to be important only in aquatic and wetland ecosystems. It was not until the advent of whole-ecosystem N budgets and the use of $^{15}N$ to trace the fate of fertilizer N in the 1950s that denitrification was found to be important in unsaturated soils. These studies suggested the importance of denitrification in fertilized agricultural soils, and with the development of the acetylene block technique in the 1970s the importance of denitrification in even forest and grassland soils was confirmed. Acetylene selectively inhibits nitrous oxide reductase (*nos*; see Fig. 13.7), allowing the assessment of $N_2$ production by following $N_2O$ accumulation in a soil core or monolith treated with acetylene. Unlike $N_2$, small changes in $N_2O$ concentration are easily detected in air.

Today, denitrification is known to be an important N cycle process wherever $O_2$ is limiting. In unsaturated soils, this frequently occurs within soil aggregates, in decomposing plant litter, and in rhizospheres. Soil aggregates vary widely in size but in general are composed of small mineral particles and pieces of organic matter $<2$ mm diameter that are glued to one another with biologically derived polysaccharides. Like most particles in soil, aggregates are surrounded by a thin water film that impedes gas exchange. Modeling efforts in the 1970s and 1980s suggested that the centers of these aggregates ought to be anaerobic owing to a higher respiratory demand in the aggregate center than could be satisfied by $O_2$ diffusion from the bulk soil atmosphere. This was confirmed experimentally in 1985 (Sexstone *et al.*, 1985), providing a logical explanation for active denitrification in soils that appeared otherwise to be aerobic, and an explanation for the almost universal presence of denitrifiers and denitrification enzymes in soils worldwide.

In addition to $O_2$, denitrification is also regulated by soil C and $NO_3^-$. C is important because most denitrifiers are heterotrophs and require reduced C as the electron donor, although as noted earlier, denitrifiers can also be chemo- and photolithotrophs. Nitrate serves as the electron acceptor and must be provided via nitrification, rainfall, or fertilizer. However, $O_2$ is the preferred electron acceptor because of its high energy yield, and thus must be depleted before denitrification occurs. In most soils the majority of denitrifiers are facultative anaerobes that will simply avoid synthesizing denitrification enzymes until $O_2$ drops below some critical threshold.

In the field $O_2$ is by far the dominant control on denitrification rates. Denitrification can be easily stimulated in an otherwise aerobic soil by removing $O_2$ and can be inhibited in saturated soil by drying or otherwise aerating it. The relative importance of C and $NO_3^-$, the other major controls, will vary by ecosystem. Under saturated conditions, such as those found in wetlands and lowland rice paddies, $NO_3^-$ limits denitrification because the nitrifiers that provide $NO_3^-$ are inhibited at low $O_2$ concentrations. Consequently, denitrification occurs only in the slightly oxygenated rhizosphere and at the sediment–water interface, places where there is sufficient $O_2$ for nitrifiers to oxidize $NH_4^+$ to $NO_3^-$, which can then diffuse to denitrifiers in the increasingly anaerobic zones away from the root surface or sediment–water interface. It is often difficult to find $NO_3^-$ in persistently saturated soils, not only because of low nitrification, but also because of the tight coupling between nitrifiers and denitrifiers. In wetlands with fluctuating water tables or with significant inputs of $NO_3^-$ from groundwater, $NO_3^-$ may be more available.

In unsaturated soils, on the other hand, the availability of soil C more often limits denitrification. In these soils C supports denitrification both directly by providing donor electrons to denitrifiers and indirectly by stimulating $O_2$ consumption by heterotrophs. It can be difficult to distinguish between these two effects experimentally; from a management perspective, there probably is no need to. It is well recognized that exogenous C stimulates denitrification, although the C added must be in an available form and must not lead to N immobilization sufficient to deplete $NO_3^-$ availability.

## OTHER NITROGEN TRANSFORMATIONS IN SOIL

Several additional microbial processes transform N in soil, although none are thought to be as quantitatively important as mineralization, immobilization, nitrification, and denitrification. *Dissimilatory nitrate reduction to ammonium* (DNRA) refers to the anaerobic transformation of nitrate to nitrite and then to ammonium. Like denitrification, this process allows for respiration to go on in the absence of $O_2$, but the ecology of DNRA is much less well understood than that of denitrification. A capacity for DNRA has been found in facultative and obligately fermentative bacteria and has long been thought to be restricted to highly anaerobic environments such as anaerobic sewage sludge bioreactors, anoxic sediments, and the bovine rumen. More recently, however, DNRA has been found to be common and important in some tropical forest soils (Silver *et al.*, 2001). In these soils the flow of inorganic N through DNRA is as large as or larger than the flow through denitrification and nitrification and may help to conserve N in these ecosystems by shunting nitrate into ammonium rather than to $N_2O$ or $N_2$. The importance of DNRA in other soils is not clear because few measurements have been made due to the difficulty of measuring DNRA in the presence of other active N-cycle transformations.

*Nonrespiratory denitrification*, like respiratory denitrification, also results in the production of N gas (mainly $N_2O$), but the reduction does not enhance growth and can occur in aerobic environments. A variety of nitrate-assimilating bacteria, fungi, and yeast can carry out nonrespiratory denitrification, which may be responsible for some of the $N_2O$ now attributed to nitrifiers in well-aerated soils (Robertson and Tiedje, 1987).

*Anaerobic ammonium oxidation* (anammox), in which ammonium and nitrite are converted to $N_2$, has been only recently discovered (Mulder *et al.*, 1995; Jetten, 2001) and thus its environmental significance is not fully known except in oceanic systems (Kuypers *et al.*, 2005). Anammox bacteria grow very slowly in enrichment culture and only under strict anaerobic conditions; anammox thus is likely to be a significant soil process only in periodically or permanently submerged soils. One pathway, involving the combination of ammonia with nitrite, has been shown to occur in three or four obligate anaerobes, including *Brocadia anammoxidans* and *Scalindua* spp. A second pathway involves the nitrifier *Nitrosomonas* spp., in a nitrogen tetraoxide ($N_2O_4$)-dependent reaction that also produces significant amounts of NO and $N_2O$ (Schmidt *et al.*, 2002).

*Chemodenitrification* occurs when $NO_2^-$ in soil reacts to form $N_2$ or $NO_x$. This can occur by several aerobic pathways. In the Van Slyke reaction, amino groups in the $\alpha$ position to carboxyls yield $N_2$:

$$RNH_2 + HNO_2 \rightarrow ROH + H_2O + N_2.$$

In a similar reaction, $NO_2^-$ reacts with $NH_4^+$, urea, methylamine, purines, and pyrimidines to yield $N_2$:

$$HNO_2 + NH_4^+ \rightarrow N_2 + 2H_2O.$$

Chemical decomposition of $HNO_2$ may also occur spontaneously:

$$3HNO_2 \rightarrow HNO_3 + H_2O + 2NO.$$

In general chemodenitrification is thought to be a minor pathway for N loss in most ecosystems. It is not easily evaluated *in situ*, however, and in the lab requires a sterilization procedure that does not itself significantly disrupt soil nitrogen chemistry.

## NITROGEN MOVEMENT IN THE LANDSCAPE

Microbial transformations of reactive N (Table 13.3) have great importance for soil fertility, water quality, and atmospheric chemistry at ecosystem, landscape, and regional scales. It is at these scales that the disconnect between what we have learned in the laboratory and what we observe in the environment (see Introduction) becomes most obvious.

One approach to thinking about microbial N cycle processes at large scales is to ask a series of questions that attempt to determine if a particular ecosystem is a source or a sink of particular N species of environmental concern (Table 13.4).

TABLE 13.3 Forms of N of Concern in the Environment

| N form | Source | Dominant transport vectors | Environmental effects |
|---|---|---|---|
| Nitrate ($NO_3^-$) | Nitrification | Groundwater | Pollution of drinking water and |
| | Fertilizer | | Coastal eutrophication |
| | Disturbance that stimulates nitrification | | |
| | Combustion (acid rain) | | |
| Ammonia ($NH_3$, $NH_4^+$) | Fertilizer | Surface runoff | Pollution of drinking water and |
| | Animal waste | Atmosphere | Eutrophication |
| Nitrous oxide ($N_2O$) | By-product of nitrification, denitrification, anammox | Atmosphere<br>Groundwater | Greenhouse gas<br>and<br>Ozone destruction in stratosphere |
| Nitric oxide (NO) | By-product of nitrification, denitrification, anammox | Atmosphere | Ozone precursor in troposphere |
| Dissolved organic N (DON) | By-product of mineralization | Surface runoff | Eutrophication (?) |
| | | Groundwater | |

Sites that are N-rich either naturally or following disturbance have a high potential to function as sources of most of the reactive N forms identified in Table 13.1 because mineralization and nitrification, the processes that produce most of these reactive forms, occur at high rates.

Nitrogen sinks are defined as habitats that have a high potential to remove reactive N from the environment, preventing its movement into adjacent ecosystems. Ecosystems such as wetlands that are wet and rich in organic materials, for example, have a great potential to function as sinks because of their ability to support denitrification. In many cases these sink areas absorb reactive N produced in source areas of the landscape. Riparian buffer zones next to streams, for example, can be managed to absorb nitrate moving out of crop fields in groundwater (Lowrance *et al.*, 1984). This nitrate can be stored in plant tissue or in soil organic matter as organic N or can be denitrified to N gas and thereby released to the atmosphere—preferably as $N_2$, a nonreactive form.

Humans have doubled the circulation of reactive N on earth, creating a nitrogen cascade in which added N flows through the environment, leading to degradation of air and water quality and coastal ecosystems in many areas (Vitousek *et al.*, 1997; Galloway *et al.*, 2003). Solutions to landscape, regional, and global N enrichment problems often rely heavily on managing microbial N transformations. For example, coastal areas of the Gulf of Mexico suffer from eutrophication

**TABLE 13.4** Criteria for Determining if a Site Is a Source or a Sink of N in the Landscape (from Groffman, 2000)

| Criteria | Determinants |
|---|---|
| Is the site N rich? | Fertilized<br>Fine texture (clay)<br>Legumes<br>Wet tropics |
| Is the site highly disturbed? | Disturbance of plant uptake (e.g., harvest)<br>Stimulation of mineralization (e.g., tillage)<br>Disturbance of links between plant and microbial processes (e.g., tillage) |
| Does the site have a high potential for denitrification? | Wet soil<br>Well-aggregated<br>High available organic matter |
| Does the site have a high potential for $NH_3$ volatilization? | High pH (>8.0) |

and hypoxia that have been linked to excess N from the Mississippi river basin (Turner and Rabalais, 1994). Proposed solutions to this problem include better management of microbial N- transformations in crop fields as well as the creation of denitrifying wetland sinks for excess N moving out of agricultural areas (Mitsch *et al.*, 2001).

Source–sink dynamics of N ultimately depend on the juxtaposition of different ecosystems in the landscape and the hydrologic and atmospheric transport vectors that link them—a complex topic that requires knowledge of hydrology and atmospheric chemistry in addition to soil ecology and microbiology. Because soil microbes play a crucial role in forming and consuming reactive N in the environment, however, their management can be an important and even crucial means for regulating N fluxes at local, regional, and global scales.

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# 14

# Biological N Inputs

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## GLOBAL N INPUTS

The nonmetallic element N is essential for life. Although a huge amount of N ($4 \times 10^{21}$ g) exists in the atmosphere, soils, and waters of Earth, more than 99% of this is in the form of $N_2$ and also is unavailable to almost all living organisms (Table 14.1). To transform $N_2$ into "reactive N," the triple bond must be broken so that N can bond with C, H, and O and form the building blocks of life. In the prehuman world, $N_2$ was transformed into reactive N via lightning discharge (5 teragrams (Tg) N year$^{-1}$; 1 Tg equals $10^{12}$ g) and by biological N fixation (BNF) (100–140 Tg N year$^{-1}$). Very few organisms possess the "N-fixing" enzyme complex necessary to carry out BNF. In the past, reactive N has not accumulated globally, because the microbial process of denitrification (whereby $NO_3^-$ is converted to $N_2$) occurs at approximately the same rate as BNF. During the past 100 years, however, the annual input of anthropogenically created reactive N has increased dramatically due to (a) modest increases in legume use in agriculture (15 to 30 Tg N year$^{-1}$), (b) large increases in fossil fuel combustion (1 Tg N year$^{-1}$ in 1860 vs

TABLE 14.1 Global Nitrogen Pool Sizes[a]

| Nitrogen pool | Pool size (g of N) |
|---|---|
| Lithosphere | $1.0 \times 10^{23}$ |
| Atmosphere | $3.9 \times 10^{21}$ |
| Coal | $1.0 \times 10^{17}$ |
| Hydrosphere | $2.3 \times 10^{19}$ |
| Soil organic N | $1.0 \times 10^{17}$ |
| Soil fixed $NH_4^+$ | $2.0 \times 10^{16}$ |
| Biota N | $3.5 \times 10^{15}$ |
| Microbial N | $1.5 \times 10^{15}$ |

[a]Reprinted from Paul and Clark (1996) by permission of the publisher.

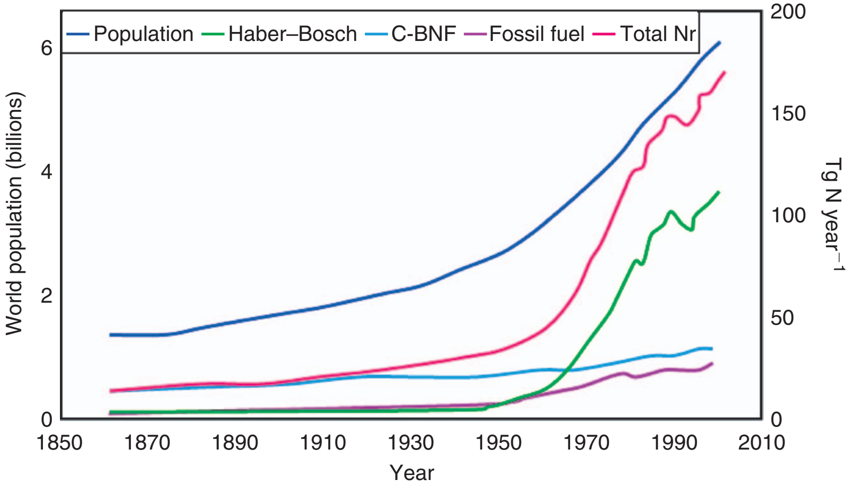



FIGURE 14.1 Global population trends from 1860 to 2000 (billions, left axis) and reactive nitrogen (Nr) creation (teragrams nitrogen (Tg N) per year, right axis). "Haber–Bosch" represents Nr creation through the Haber–Bosch process, including production of ammonia for nonfertilizer purposes. "C-BNF" (cultivation-induced biological nitrogen fixation) represents Nr creation from cultivation of legumes, rice, and sugarcane. "Fossil fuel" represents Nr created from fossil fuel combustion. "Total Nr" represents the sum created by these three processes. Reproduced from Galloway *et al.* (2003); copyright American Institute of Biological Sciences.

25 Tg N year$^{-1}$ in 2000), and (c) enormous increases in the use of Haber–Bosch process-derived fertilizer N for food production (zero, pre-20th century; currently ~110 Tg N year$^{-1}$) (Fig. 14.1). The input of reactive N has increased over 10-fold in 100 years, with about 85% being used by agriculture to support the ever-increasing human population during the same period. In addition, enhanced use of reactive N for food production has had secondary effects on the N cycle. For example, the intensification of agriculture and its expansion into forest and grasslands has released

reactive N from long-term storage in soil organic matter. It has been estimated that the burning of forests and grasslands, draining of wetlands, and soil tillage liberate approximately 40 Tg N year$^{-1}$ of reactive N (Vitousek *et al.*, 1997).

Although yearly estimates of global BNF have not changed much across both pre- and postindustrial eras, the distribution of BNF has changed as a result of urbanization and intensive agriculture. Large areas of diverse natural vegetation, which once included $N_2$-fixing species as part of their floral composition, have been replaced by monocultures of non-$N_2$-fixing, high-yielding crop species that require addition of reactive N to achieve their yield potentials. In contrast, agricultural BNF is restricted to smaller areas of intensively managed crop and grazing lands. Unfortunately, increased use of $N_2$-fixing grain legumes for human and domestic animal consumption often results in net export of N from agricultural soils. Another major consequence of the human-driven alteration of the N cycle involves the movement of reactive N from sites of application to remote sites. BNF occurs inside living organisms in which fixed N is quickly assimilated into cell constituents. In contrast, reactive N applied to non-$N_2$-fixing crop species as fertilizer or animal waste has a less certain fate. For example, ammoniacal and urea-based N fertilizers can be converted to various gaseous or water-soluble N oxides ($NO_x$) by the microbial processes of nitrification and denitrification and dispersed from the site of application. $NH_3$ can also be dispersed to the atmosphere for deposition elsewhere (a) during application of anhydrous $NH_3$ or animal waste or (b) after enzymatic conversion of soil-applied urea to $NH_3$ by the enzyme urease. There are both positive and negative effects associated with the deposition of reactive N at sites remote from its source. Plant and microbe growth may be stimulated and N immobilized. Scientists have estimated that an extra 100–1000 Tg C year$^{-1}$ may be fixed globally because of atmospheric transfer and remote deposition of reactive N. Too much N deposition, however, can result in soil acidification, greater $NO_3^-$ leaching, and loss of plant species diversity in terrestrial ecosystems.

It might seem somewhat ironic that the justification for studying BNF so intensively over the past 100 years lies in the need to increase inputs of reactive N via BNF into N-limited agroecosystems. These studies continue, however, against the backdrop of negative environmental consequences caused by the huge global excesses of reactive N. Nonetheless, scientists work to thoroughly understand the mechanism of BNF in order to increase the efficiency of manufacturing N fertilizer. In addition, there is an urgent need to learn how certain $N_2$-fixing plants and specific microorganisms establish symbiotic and endophytic associations while others do not. There is hope that one day scientists might be able to create $N_2$-fixing corn, wheat, and rice and reduce the use of fertilizer N.

## BIOLOGICAL NITROGEN FIXATION

BNF is a process exclusively restricted to the prokaryotes of the domains Archaea and Bacteria. However, there are many examples of symbiotic associations that

TABLE 14.2 Examples of Genera of Diazotrophic Bacteria Arranged by Mode of Energy Generation and the Oxygen Sensitivity of Their Diazotrophy

| Energy source | Sensitivity of $N_2$ fixation to oxygen | Examples (Genera) |
|---|---|---|
| Heterotrophic | Aerobic diazotrophs | *Azotobacter*, *Gluconacetobacter* |
| | Microaerophilic diazotrophs | *Azospirillum*, *Herbaspirillum*, *Methylococcus* |
| | Facultatively aerobic diazotrophs | *Klebsiella*, *Paenibacillus*, *Enterobacter* |
| | Obligately anaerobic diazotrophs | *Clostridium*, *Desulfovibrio*, *Methanosarcina* |
| Phototrophic | Aerobic diazotrophs (primarily filamentous heterocyst-forming cyanobacteria) | *Anabaena*, *Nostoc* |
| | Microaerophilic diazotrophs (filamentous nonheterocystous cyanobacteria) | *Lyngbya*, *Oscillatoria* |
| | Facultatively aerobic diazotrophs (purple nonsulfur bacteria) | *Rhodobacter* |
| | Obligately anaerobic diazotrophs (purple sulfur bacteria) | *Chromatium* |

Adapted from Young (1992) by permission of the publisher.

have developed between eukaryotes and prokaryotes that circumvent the eukaryotes' needs for an exogenous supply of fixed N. Bacteria that use $N_2$ as the sole source of N are called diazotrophs. Although only a limited number of bacterial species fix $N_2$, they represent a wide variety of phylogenetically and physiologically distinct types that occupy different ecological niches (Table 14.2). These bacteria use diverse energy sources including sunlight (phototrophs), reduced inorganic elements and compounds (lithotrophs), and a plethora of different organic substrates (heterotrophs). They are represented by obligate aerobes and facultative and obligate anaerobes. This metabolic diversity indicates that diazotrophs can contribute fixed N to other life forms in a wide variety of environments.

BNF is mediated by an enzyme complex called nitrogenase, which is composed of two proteins (dinitrogenase and nitrogenase reductase). There are three different forms of nitrogenase that differ in their requirements for molybdenum (Mo), vanadium (V), or iron (Fe) as a critical metallic component of the cofactor associated with the catalytic site. Most of the nitrogenases that have been studied extensively contain a Mo cofactor. $N_2$ fixation is an energetically expensive process. Two ATP molecules are required for each electron transferred from nitrogenase reductase to dinitrogenase, which contains the catalytic site. Nitrogenase reductase is recharged with electrons provided by a protein called ferredoxin or flavodoxin. A total of 16ATP molecules are required to provide the six electrons necessary to reduce $1N_2$ molecule into $2NH_3$ because 25% of the energy used to reduce $N_2$ is "lost" in the reduction of $2H^+$ to $H_2$. One mole of $H_2$ is formed per mole of $N_2$

TABLE 14.3 Energy Costs for Biological $N_2$ Fixation Relative to Assimilation of $NH_4^+$-N[a]

| Bacterial species | Energy source | $N_2$-N fixed/g C source used (mg) | $NH_4^+$-N assimilated/g C source used (mg) |
|---|---|---|---|
| Anaerobic growth: *Clostridium pasteurianum* | Sucrose | 11 | 22 |
| Microaerophilic growth: *Azospirillum brasilense* | Malate | 26 | 48 |
| Aerobic growth: *Azotobacter vinelandii* | Sucrose | 7 | 38 |

[a]Adapted from Hill (1992) by permission of the publisher.

transformed to 2 $NH_3$. Considerable interest has been shown in those diazotrophs that contain an enzyme called uptake hydrogenase, which reoxidizes $H_2$ to protons and electrons and salvages the reductant lost in nitrogenase-dependent $H_2$ formation.

The high energy costs of BNF can also be illustrated by comparing the amount of reduced C oxidized $g^{-1}$ of N assimilated from either $N_2$ or $NH_4^+$-N (Table 14.3). It can be seen that the cost of assimilating $N_2$-N is about twice that of assimilating $NH_4^+$-N. Several factors contribute to this high energy cost. In addition to the high ATP cost of reducing $N_2$ to $NH_3$, at least 20 genes and their products are needed for synthesis of a completely functional $N_2$-fixing enzyme system (Table 14.4). The genes involved collectively in synthesis of nitrogenase and the catalytic process of $N_2$ fixation are called *nif* genes. Accessory genes are called *fix* genes, and they are also necessary for the function and regulation of nitrogenase in micro-aerobic and aerobic diazotrophic bacteria. The catalytic process of $N_2$ fixation is remarkably slow and inefficient. The two proteins must come together and dissociate eight times to reduce one molecule of $N_2$ to two $NH_3$. To compensate for this sluggish mechanism, nitrogenase might account for ~10% of total cell protein in a diazotrophic microorganism. Finally, many of the *nif* gene proteins are denatured by oxygen and turnover quickly in an aerobic environment. Replacement of these denatured proteins constitutes an additional energy expense.

Because of the extreme sensitivity of nitrogenase to irreversible denaturation by $O_2$, aerobic $N_2$-fixing bacteria have developed various $O_2$ protection mechanisms. For example, some diazotrophic $O_2$-evolving cyanobacteria produce specialized cells called heterocysts under $N_2$-fixing conditions (Fig. 14.2). Heterocysts do not divide like their neighboring vegetative cells, do not evolve $O_2$ or fix $CO_2$, and use light energy to provide the energy needed for BNF. A combination of respiration and thick cell walls excludes $O_2$ sufficiently well to protect the dinitrogenase enzyme complex. In some $N_2$-fixing nonheterocystous cyanobacteria, temporal separation of $N_2$ fixation and $O_2$ evolution results in BNF occurring primarily during darkness in the absence of $O_2$ production. At least under C-rich laboratory conditions, aerobic, $N_2$-fixing bacteria such as *Azotobacter* express a high respiratory rate that

TABLE 14.4 Nitrogen-Fixing (*nif*) Genes and Their Products

| Gene | Product and function |
|---|---|
| *nif* H | Nitrogenase reductase subunit; binds two molecules of MgATP and reduces nitrogenase by single electron transfer |
| *nif* D | Nitrogenase protein α subunit of dinitrogenase |
| *nif* K | MoFe protein β subunit of dinitrogenase |
| *nif* F | Flavodoxin, reductant of nitrogenase reductase |
| *nif* J | Pyruvate–flavodoxin oxidoreductase; couples oxidation of pyruvate to reduction of flavodoxin |
| *nif* S | Pyridoxal-dependent cysteine desulfurase; required for synthesis of active dinitrogenase and nitrogenase reductase |
| *nif* U | Complements *nif* S; required for mobilization of Fe and S for metallocluster assembly and synthesis of active enzyme |
| *nif* V | Homocitrate synthase; organic component of FeMo cofactor |
| *nif* N | Subunit of *nif* $N_2E_2$, which provides a transient site for the assembly of FeMo cofactor |
| *nif* E | Subunit of *nif* $N_2E_2$ |
| *nif* B | FeMo cofactor precursor biosynthesis |
| *nif* Q | Early step in FeMo cofactor biosynthesis |
| *nif* X | Intermediate carrier in FeMo cofactor biosynthesis |
| *nif* Y | Intermediate carrier in FeMo cofactor biosynthesis |
| *nif* W | Required for synthesis of fully active dinitrogenase |
| *nif* Z | Required for synthesis of fully active dinitrogenase |
| *nif* A | Positive regulatory protein |
| *nif* L | Negative regulatory protein |
| *nif* T | Unknown function |
| *naf* Y | Probably an intermediate carrier in FeMo cofactor biosynthesis |

Modified from Dean and Jacobsen (1992) by permission of the publisher.

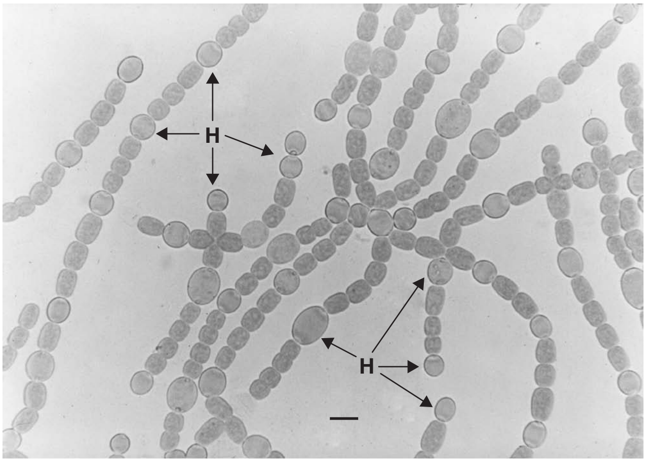



FIGURE 14.2 $N_2$-fixing heterocystous cyanobacteria recovered from the water fern *Azolla.* Heterocysts are designated with the letter "H". Note the high percentage of heterocysts relative to vegetative cells. This reflects a high rate of $N_2$ fixation by the cyanobacterium in the symbiotic state with the *Azolla.* Courtesy of J. C. Meeks; used by permission.

maintains a low $O_2$ concentration inside the cell. If the $O_2$ level increases quickly, however, nitrogenase interacts with other redox-sensitive proteins in the cell that invoke a conformational change that inactivates nitrogenase and prevents its irreversible damage.

Because $NH_3$, the primary product of $N_2$ fixation, is toxic to cells in high concentrations, $N_2$-fixing bacteria possess highly efficient mechanisms of $NH_3$ assimilation that prevent its accumulation. In most diazotrophs the enzyme combination of glutamine synthetase (GS) and glutamate synthase (GOGAT) carries out this task (Eqs. (1) and (2)):

$$\text{Glutamate} + NH_3 + \text{ATP} \xrightarrow{\text{Glutamine synthetase}} \text{Glutamine} + \text{ADP} + P_i \quad (1)$$

$$\text{Ketoglutarate} + \text{Glutamine} + \text{NADH} \xrightarrow{\text{GOGAT}} 2\ \text{Glutamine} + \text{NADox} \quad (2)$$

As can be seen both ATP and reductant are required at this stage of $NH_3$ assimilation and add an additional energy burden to the $N_2$-fixing organism. Because BNF is energetically expensive, bacteria do not usually fix $N_2$ in the presence of reactive N sources. A number of mechanisms have been identified whereby nitrogenase activity is inhibited, and *nif* gene expression is down-regulated in response to increasing levels of $NO_3^-$, $NH_4^+$, and/or amino acid N in the environment (Merrick, 1992).

Many attempts have been made to quantify BNF in both natural and agricultural ecosystems, and the values vary widely (Table 14.5). Two methods are generally

TABLE 14.5 Range of Rates of BNF Measured under Field Conditions by Different Diazotrophic Organisms and Their Associations

| Diazotrophic bacteria and their associations | $N_2$ fixed (kg $ha^{-1}$ $year^{-1}$) |
|---|---|
| Free-living bacteria (associated with wood decay, straw decomposition, cyanobacterial mats) | <1–10 |
| Examples of plant–cyanobacterial associations | |
| Cryolithic crusts | 10–80 |
| *Azolla* | ≤300 |
| Examples of legume–rhizobial associations | |
| Soybean | 60–115 |
| Beans | 50–100 |
| Alfalfa | 130–250 |
| White clover | 200 |
| Examples of nonlegume–*Frankia* associations | |
| Alder | 50–300 |
| *Ceanothus* | 50–60 |
| *Hippophae* | 10–60 |

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favored: (a) the acetylene reduction method and (b) incorporation of the stable isotope of nitrogen, $^{15}N$. Like many other metalloenzymes that utilize gaseous substrates, nitrogenase has a broad substrate range and will reduce many small triple-bonded molecules, including acetylene. In the 1960s, it was discovered that the enzyme nitrogenase reduced acetylene to ethylene ($CH_2 = CCH_2$), which can be easily measured by gas chromatography. This method was much easier to use than the traditional method of quantifying the incorporation of $^{15}N_2$ into bacterial or plant biomass. Although the advent of acetylene reduction methodology accelerated progress in our understanding of BNF, acetylene reduction is limited in its ability to accurately quantify $N_2$ fixed in the field over a growing season, and the stoichiometry of $C_2H_2$:$N_2$ reduced varies with environmental conditions. Indeed, most soil microbiologists and field scientists generally favor the use of $^{15}N$ for quantifying $N_2$ fixation under field conditions (Weaver and Danso, 1994). Because $^{15}N_2$ is expensive and awkward to handle under field conditions, the isotope dilution approach is preferred.

The N in biological systems is composed predominantly of two stable isotopes, $^{15}N$ and $^{14}N$, which constitute about 0.3663 and 99.6337%, respectively, of the N in the atmosphere. A $N_2$-fixing organism will incorporate $^{14}N$ and $^{15}N$ from an N source in proportion to the concentrations of the two isotopes in that source. If one of these N sources is atmospheric $N_2$, and because atmospheric $N_2$ generally has a lower percentage of $^{15}N$ than other sources (soil or fertilizer N, for example), the proportion of soil or fertilizer $^{15}N$ assimilated by the $N_2$ fixer will be "diluted" by the relative contribution of $N_2$ to the cell N budget. In general, the "isotope dilution" approach to measuring BNF under soil conditions involves the addition of a small amount of $^{15}N$-enriched inorganic N to the soil to increase the $^{15}N/^{14}N$ ratio of available soil N. In principle, the $^{15}N/^{14}N$ ratio of organisms that assimilate soil N will be the same as the $^{15}N/^{14}N$ ratio of the available soil N. In the case of an organism that is fixing atmospheric $N_2$, and assimilating soil N, its $^{15}N/^{14}N$ ratio will be lowered proportional to the amount of $N_2$ being fixed; i.e., the $^{15}N$ content of the biomass will be "diluted" as a result of BNF. The percentage of N derived from the atmosphere (%Ndfa) is represented by the following equation:

$$\%\text{Ndfa} = 1 - \frac{\text{atom}\%^{15}\text{N excess in N}_2\text{-fixing plant}}{\text{atom}\%^{15}\text{N excess in nonfixing plant}} \times 100.$$

## FREE-LIVING $N_2$-FIXING BACTERIA

Diazotrophic prokaryotes can be divided into those that carry out BNF in a symbiotic or commensalistic relationship with a eukaryote and those that fix $N_2$ in a free-living state. Because the major factor usually limiting BNF is the C/energy supply, it is reasonable to predict that free-living photosynthetic diazotrophs would fix greater amounts of $N_2$ under some soil conditions than free-living heterotrophs

and that the latter would fix measurable amounts of $N_2$ only in the presence of readily available plant-derived C, e.g., labile C in the rhizosphere zone of an actively growing plant, or during the decomposition of herbaceous and woody plant residues of high C:N ratio. Facultative and obligately anaerobic diazotrophic bacteria are often found in decaying wood, where it is assumed that cellulolytic and ligninolytic fungi depolymerize the sugars and phenolics necessary to support diazotrophs (Sylvester and Musgrave, 1991). A similar situation exists in agricultural straw residues, in which it has been shown that additions of both cellulolytic and diazotrophic bacteria enhance BNF and accelerate the decomposition of the N-deficient straw. It remains extremely difficult to quantify the contribution of BNF by free-living diazotrophs to an ecosystem, even in environments in which BNF can be detected by the acetylene reduction method. Estimates of free-living BNF in soil and plant residue-enriched environments usually range between $<1$ and 10 kg N $ha^{-1}$ $year^{-1}$, with many natural systems occurring in the lower range of these estimates.

## ASSOCIATIVE $N_2$-FIXING BACTERIA

Root secretions and other rhizodepositions are a major source of plant C input to soil, and diazotrophic bacteria are associated with the roots of plants (Reinhold-Hurek and Hurek, 1998). During the last third of the 20th century, many studies demonstrated that a variety of diazotrophic bacteria associate with the roots of tropical grasses, notably *Paspalum* and *Digitaria* species, where they fix measurable amounts of $N_2$. These bacteria belong to the genera *Azospirillum*, *Herbaspirillum*, and *Burkholderia.* They are found both in the rhizosphere and in the intercellular spaces of the root cortex. It has been claimed these bacteria can provide between 5 and 30% of the total N accumulated by the plants. Using the $^{15}N$ isotope dilution technique, Urquiaga *et al.* (1992) showed that some Brazilian sugarcane cultivars could derive $>60\%$ of plant N from BNF. A diazotrophic bacterium, *Gluconacetobacter diazotrophicus*, was isolated from sugarcane, found to occupy internal tissues at high cell densities ($10^6$ to $10^7$ cells $g^{-1}$) (Fig. 14.3), and could grow on high sucrose concentrations (10%) and fix $N_2$ optimally at pH 5.5. Kennedy *et al.* (2000) showed that a cultivar of sugarcane (SP70-1143) inoculated with a wild-type strain of *Glu. diazotrophicus* and grown under N-limiting conditions produced a higher biomass and contained more total N than plants inoculated with a *nif*$^-$ mutant (cannot fix $N_2$) or that were not inoculated (Table 14.6). When N was not limiting, however, both the wild-type and the *nif*$^-$ mutant stimulated greater growth and total N content of the plants. Several questions remain unanswered. Although $^{15}N_2$ was incorporated into plants inoculated with wild-type *Glu. diazotrophicus*, it is unclear what portions of the fixed N remain associated with the bacteria versus being transferred to plant tissues. In addition, it still remains unclear to what extent sugarcane growth enhancement can be attributed to BNF or to improved sequestration of soil N brought about by production of plant growth hormone-like compounds by *Glu. diazotrophicus.*

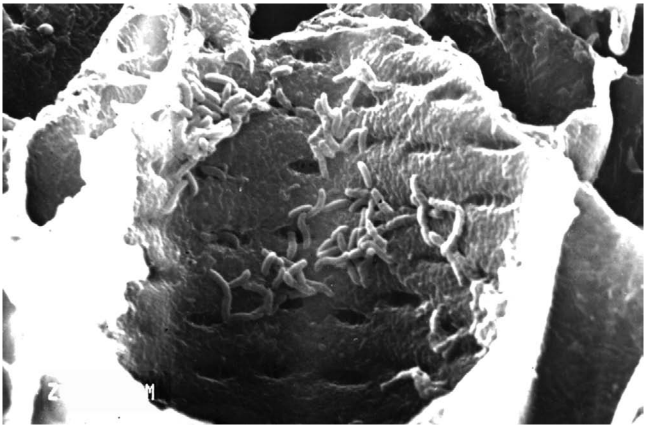


FIGURE 14.3 Scanning electron micrograph of *Herbaspirillum seropedicae* within the metaxylem of a sugarcane stem. Note that the bacteria are associated closely with the walls of the vessel. Courtesy of F. Olivares; used by permission.

TABLE 14.6 Plant Dry Weight and Total N Content of Sugarcane Plants Inoculated with *Gluconacetobacter* PA15 and a Nondiazotrophic Mutant, Mad3A

| Treatment | Total plant dry weight (g) | Total N content (mg) |
|---|---|---|
| Minus fertilizer N | | |
| Uninoculated | 5.3[d] | 23.3[d] |
| +PA15 | 8.2[c] | 46.1[c] |
| +Mad3A | 6.1[d] | 30.7[d] |
| With fertilizer N | | |
| Uninoculated | 18.1[b] | 116.9[b] |
| +PA15 | 28.3[a] | 160.9[a] |
| +Mad3A | 23.2[a] | 143.2[a] |

Measurements were made 60 days post-inoculation of plants. Reproduced from Kennedy *et al.* (2000) by permission of the publisher.

Values followed by different superscript letters are statistically significantly different at $p < 0.05$.

## PHOTOTROPHIC BACTERIA

Because BNF is an energy-expensive process, it is not surprising that photosynthetic microorganisms are major suppliers of newly fixed N in certain soil ecosystems. In rice paddies, for example, cyanobacteria and other photosynthetic bacteria provide substantial inputs of N because of the flooded soil conditions early in the growing season, the presence of abundant light until canopy closure, adequate phosphate fertilization, and the low $O_2$ conditions found at the sediment–water interface. There is a long history of BNF in rice production in Asia through use of the water fern *Azolla* and its cyanobacterial microsymbiont, *Anabaena azollae*, either as a green manure or in coculture with the rice crop. N inputs of $>100$ kg N ha$^{-1}$ year$^{-1}$

have been recorded (Wagner, 1997). In sharp contrast to the tropical rice paddy ecosystem, biological soil crusts are highly specialized photosynthetic communities of cyanobacteria, algae, lichens, and mosses that are commonly found in arid and semiarid environments throughout the world (Belnap and Lange, 2003). Because they are concentrated in the top few millimeters of soil, biological crusts contribute to soil stability and water infiltration as well as soil N status. Estimates of annual BNF rates in biological soil crusts have ranged widely from 1 to 350 kg N $ha^{-1}$ $year^{-1}$, but generally fall in the range of 10 kg N $ha^{-1}$ $year^{-1}$. Because of rapid wetting and drying, the $N_2$ fixed tends to be released quickly and provides immediate benefit to the vascular plants in the surrounding ecosystem.

## SYMBIOTIC N2-FIXING ASSOCIATIONS BETWEEN LEGUMES AND RHIZOBIA

Most terrestrial $N_2$-fixing symbioses involve a $N_2$-fixing prokaryote and a photosynthetic host. Because the prokaryote gains its energy from the photosynthetic host, the energy cost of BNF is compensated adequately, and considerable amounts of N can be fixed if other factors are not limiting (Table 14.5). In agroecosystems, an intensively managed perennial legume, such as alfalfa (*Medicago sativa*), can fix several hundred kilograms of N per hectare per year. Legumes are the most widely recognized $N_2$-fixing symbioses because of their importance as a food source. In 1886, Hellreigel and Willfarth demonstrated the ability of legumes to convert $N_2$ into organic N. Quickly thereafter, Beijerinck in 1888 isolated bacteria (rhizobia) from legume root nodules and showed they were able to reinfect the legume, form nodules, and fix $N_2$ in the symbiosis. Studies carried out in the early part of the 20th century illustrated that rhizobia recovered from root nodules of different legume species expressed different phenotypic characteristics and expressed plant host specificity. Today we recognize that rhizobia fall into several genera and species within the Alphaproteobacteria and are currently subdivided into six genera and >20 species (Table 14.7).

### FORMATION OF THE SYMBIOSIS

The establishment of the mutualistic relationship between rhizobia and legumes is accomplished via a series of developmental stages, all of which are mediated by a chronological cascade of physiological signals from both participants. The process of establishing the symbiotic relationship is highly specific (i.e., a specific bacterial species with one, or a limited number of, legume species). At each stage of the formation of this relationship, the chemical signals released by the plant and the bacterium reciprocally induce unique genetic programs that lead to the formation of a nodule and result in BNF.

*Stage 1.* Rhizobia exist primarily as soil saprophytes that are widely distributed and are found in the rhizospheres of plant roots. They initiate nodule formation by

**TABLE 14.7** Genera and Species of the Root Nodule Bacteria of Legumes and Examples of Their Hosts

*Allorhizobium*
*A. undicola* (*Neptunia*)
*Azorhizobium*
*A. caulinodans* (*Sesbania*)
*Bradyrhizobium*
*B. canariense* (genistoid legumes)
*B. japonicum* (soybean)
*B. elkanii* (soybean)
*B. liaoningense* (soybean)
*B.* spp. (*Vigna, Lupinus,* etc.)[a]
*Mesorhizobium*
*M. amorphae* (*Amorpha*)
*M. chacoense* (*Prosopis*)
*M. ciceri-* (*Cicer*, chickpea)
*M. huakii-* (*Astragalus*, milkvetch)
*M. loti-* (*Lotus*, trefoil)
*M. mediterraneum* (*Cicer*, chickpea)
*M. plurifarium* (tropical trees)
*Rhizobium*
*R. etli* (bean, *Phaseolus*)
*R. galegae* (*Galega*)
*R. gallicum* (bean, *Phaseolus*)
*R. giardinii* (bean)
*R. huakuii* (*Astragalus*)
*R. huautlense* (*Sesbania*)
*R. indigofera* (*Indigofera*)
*R. leguminosarum* (three biovars nodulate (i) clovers; (ii) peas, lentils, and vetch; (iii) bean
*R. mongolense* (*Medicago*)
*R. tropici* (bean, *Phaseolus, Leucaena*)
*Sinorhizobium*
*S. americanum* (*Acacia*)
*S. arboris* (tree legumes)
*S. fredii* (soybean)
*S. kostiense* (tree legumes)
*S. kummerowiae* (*Kummerowia*)
*S. medicae* (annual medics)
*S. meliloti* (alfalfa)
*S. morelense* (*Leucaena*)
*S. saheli* (*Sesbania*)
*S. teranga* (*Sesbania*)

[a]Although many legumes are nodulated by bacteria of the *Bradyrhizobium* genus, the latter have not received official species designation. They are referred to by the name of the legume host from which they were isolated (e.g., *Bradyrhizobium* [*Lupinus*]).

penetrating a legume root either by infection of root hairs (e.g., clover and pea) or, more rarely, by entry at the sites of lateral root emergence (peanut) or through penetration of root primordia found on the stems of some legumes such as *Sesbania* and *Aeschynomene.* In the case of root-hair infection, the bacteria attach

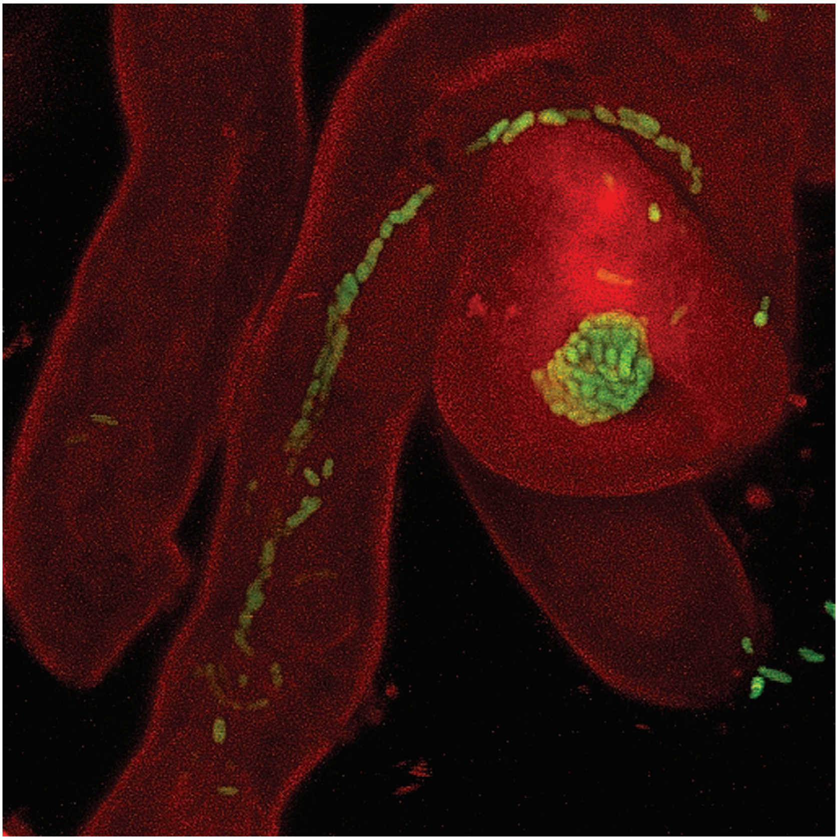


FIGURE 14.4 *Medicago* root hair curling and infection thread invasion by rhizobia upon inoculation with *Sinorhizobium meliloti.* Courtesy of Rene Geurts; used by permission.

to the cell wall of the root hair and trigger a series of morphological and physiological changes in the latter. Inhibition of cell expansion on one side of the root hair causes it to curl back on itself with the rhizobia attached to the inside surface of the curled root hair wall.

*Stage 2.* Rhizobia pass down the root hair inside a structure known as an infection thread (or tube), which is an invagination of the root hair wall and is clearly visible within 2 days of seedling exposure to rhizobia (Fig. 14.4). The infection thread branches and penetrates into the root cortex, where cortical cells divide and enlarge to form a prenodule in response to the rhizobial invasion.

*Stage 3.* As the infection thread gets near the dividing cortical cells it forms various side branches, each of which eventually comes to a halt at the cell wall of a cortical cell. At the point of contact, the cell wall dissolves and the rhizobia (10–100 bacterial cells) are delivered into the nodule cell by endocytosis.

*Stage 4.* Rhizobia are released from the infection thread into root cortical cells and are enclosed within a plant-derived membrane called the peribacteroid membrane. They remain physically isolated from the host cell cytoplasm. Each membrane-enclosed bacterium is referred to as a "symbiosome." As the plant cortical cells divide and become infected to form the nodule structure, the vascular strands of the plant extend into the nodule to permit exchange of nutrients.

*Stage 5.* The rhizobia undergo further pleiomorphic and biochemical changes in the symbiosome and ultimately commence BNF.

Root nodules differ in appearance and structure depending upon the host plant species. Some plants develop determinate nodules that are generally spherical in shape, reach a finite size, and possess no active meristems, e.g., *Phaseolus* (beans), *Vigna* (cowpea), and *Glycine* (soybean). Other legumes produce cylindrically shaped, indeterminate nodules with persistent active meristems, which enable the nodule to grow, enlarge, and produce new cortical cells in response to plant growth, e.g., *Medicago* (alfalfa), *Trifolium* (clover), *Vicia* (vetch). In indeterminate nodules, nonbacteroid rhizobia remain in the infection threads and they infect new cortical cells as the meristem of the nodule continues to divide.

## RHIZOBIAL NODULATION GENES

The rhizobial genes needed to establish a symbiosis have been identified, and some are listed (Table 14.8). Genes referred to as common nodulation genes (*nod* A, B, C, and D) are found in all rhizobia and are essential for initiation of nodulation. In addition, many other genes that define the legume host range of specific rhizobia have been identified and are referred to as *nod*, or *nol*, or *noe* genes. These genes are involved in the synthesis and transport of an unusual class of compounds referred to as "*nod* factors" that induce nodule formation. *Nod* factors are composed of an oligosaccharide backbone of β1-4-linked *N*-acetylglucosamine residues with a fatty acid acyl group attached to the N atom of the nonreducing acetyl glucosamine residue (Fig. 14.5). These compounds are referred to as lipochitin oligosaccharides or LCOs. LCOs usually contain three to six *N*-acetylglucosamine residues. The common *nod* genes A, B, and C encode enzymes that play key roles in synthesis of the LCO backbone structure and include chitin oligosaccharide synthase (*nod*C), chitin oligosaccharide deacetylase (*nod*B), and acyltransferase (*nod*A). A large number of structural variants exist among the LCOs (Table 14.9). Host specificity is based upon the structural variations among the *nod* factors. A number of specific substituents ("decorations") are found on positions R1 through R9, and different rhizobial genes (referred to as host-range genes) are involved in the attachment of these moieties. For example, the placement of a $SO_4$ group at position R5 of the LCO from *Sinorhizobium meliloti* is essential for nodulation of alfalfa (*M. sativa*), whereas *O*-methyl fucosylation of R5 is essential for nodulation of soybean (*Glycine max*) by *Bradyrhizobium japonicum. Nod* factors are produced by rhizobia in response to inducers secreted by germinating seedlings and by plant roots. The most potent inducers are flavones

**TABLE 14.8** Examples of Nodulation (*nod*) Genes and Their Proposed Functions[a]

| Gene | Proposed function |
|---|---|
| Regulatory genes | |
| *nod*$D_1$ | Transcriptional activator |
| *nod*V | Two-component regulator |
| *nod*W | Two-component regulator |
| *nol*A | Transcriptional regulator |
| *nol*R | Repressor, DNA binding protein |
| *syr*M | Transcriptional regulator |
| *Nod* factor core synthesis | |
| *nod*A | Acetyltransferase |
| *nod*B | Deacetylase |
| *nod*C | Chitin synthase |
| *nod*M | D-Glucosamine synthase |
| *Nod* factor core modifications | |
| *nod*E | Ketoacyl synthase |
| *nod*F | Acyl carrier protein |
| *nod*H | Sulfotransferase |
| *nod*L | Acetyl transferase |
| *nod*S | Methyl transferase |
| *nod*L | ATP-sulfurylase |
| *nod*P | ATP-sulfurylase |
| *nod*Z | Fucosyl transferase |
| *nol*L | *O*-acetyl transferase |
| *nol*O | Carbamoyl transferase |
| *noe*C | Arabinosylation |
| *noe*I | 2-*O*-methylation |
| *Nod* factor transport | |
| *nod*I | Integral membrane protein |
| *nod*J | Outer membrane transport |
| *nod*O | Pore-forming protein |

[a]The list of genes is not inclusive; some genes coding for the same function in different organisms are coded differentially.

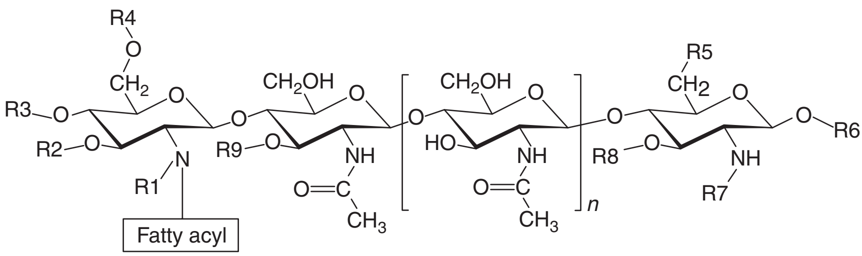



**FIGURE 14.5** General structure of the *nod* factors produced by rhizobia. The presence of substituents numbered R1–R9 is variable among strains of rhizobia. See Table 14.9 for examples of these substituents. In the absence of substituents, R groups stand for hydrogen (R1), hydroxy (R2, R3, R4, R5, R6, R8, and R9), and acetyl (R7). Courtesy of H. Spaink; used by permission.

TABLE 14.9 Examples of Modifications of the Rhizobial Core *Nod* Factors and the Genes and Their Products Involved[a]

| Bacterium | Core substituents | Genes |
|---|---|---|
| *S. meliloti* | R4:Ac, R5:S, C16:2 | R4:*nod*L, R5:*nod*H, fatty acid:*nod*AFEG |
| *M. loti* | R1:Me, R3:Cb, R5:AcFuc | R1:*nod*S, R3:*nol*O, R5:*nod*Z and *nol*L |
| *B. japonicum* | R5:MeFuc | R5:*nod*Z and *noe*I |
| *A. caulinodans* | R1:Me, R4:Cb, R5:Fuc, R8:Ara | R1:*nod*S, R4:*nod*U, R5:*nod*Z, R8:*noe*C |

[a]These examples do not represent *nod* factors produced by all species of a named genus nor all strains of the same species.

and isoflavones, which are phenolic compounds collectively referred to as flavanoids. The flavanoids bind to the *nod*D gene product, which acts as a transcriptional activator of the other *nod* genes.

Different types of flavanoid molecules are involved in *nod* gene induction in different legume species, for example, luteolin in *M. sativa* (alfalfa) and genistein in *Gly. max* (soybean).

## PLANT NODULATION GENES

Several plant genes (referred to as *nodulin* genes) that are exclusively involved in the early stages of root-hair infection and nodule development have been identified (Radutolu *et al.*, 2003; Limpens *et al.*, 2003). Other nodulins are expressed at later stages of nodule functional development and include hemoglobin and enzymes involved in N assimilation. Recent analysis of plant mutants has placed emphasis on identifying the early *nodulin* genes. For example, *NFR*1/*NFR*5 are genes placed at the top of the hierarchical cascade because plants with mutations in these genes lack all known responses to *nod* factor. *NFR*1/*NFR*5 code for a transmembrane receptor-like kinase (an enzyme that acts as a "molecular switch" by covalently attaching phosphate groups to other proteins), which turns enzymatic pathways on or off. It is thought that a specific part of the kinase protein binds to the *N*-acetylglucosamine backbone of *nod* factor and initiates the nodulation process (Fig. 14.6). In the legume *Medicago truncatula*, mutants have been obtained in three other genes (*DMI*1 and *DMI*2 and *DMI*3) that do not exhibit most *nod* factor responses (no root-hair branching, *nodulin* gene expression, or cortical cell division). While root hairs in mutants *DMI*1 and *DMI*2 have lost their ability to undergo oscillations in intracellular Ca levels in response to *nod* factor, Ca oscillations are unimpaired in root hairs of the *DMI*3 mutant, suggesting that its role in nodulation is farther downstream from *DMI*1 and *DMI*2. This work suggests there is a relationship between *DMI*1 and *DMI*2 and Ca "spiking" associated with root hair infection and that *DMI*3 is involved in relaying this Ca signal to other parts of the nodule-forming process. Interestingly, mutants *DMI*1, *DMI*2, and *DMI*3 cannot form mycorrhizal associations with the arbuscular mycorrhizal (AM) fungus

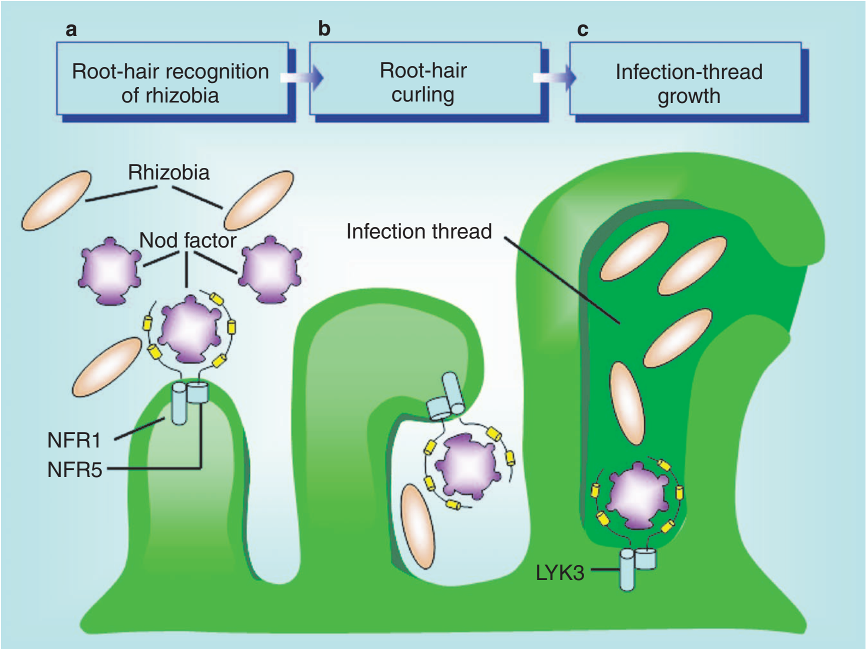



FIGURE 14.6 Proposed model of how rhizobial *nod* factor interacts with the early nodulin proteins NFR1 and NFR5 to promote root-hair curling. LYK3 is hypothesized to be involved in infection-thread growth. Reproduced from Parniske and Downie (2003) by permission of the publisher and the authors.

*Glomus*, implying there are common molecular signals for root infection of plants by AM fungi and rhizobia. The temporal spectrum of physiological and genetic changes occurring during nodule organogenesis is just beginning to be understood. The emerging picture seems to indicate that *nod*-signal activities result in changes of endogenous phytohormone levels and changes in the sensitivity of specific plant tissues to the actions of these phytohormones.

## DEVELOPMENT OF BNF AND NITROGEN ASSIMILATORY PROCESSES IN NODULES

Most of the bacterial genes that are specifically induced in bacteroids appear to be regulated, at least in part, by $O_2$. This is not surprising since, as mentioned earlier, the nodule structure and function are designed to provide a microaerobic environment for BNF. The outer cortex of the nodule is aerobic but beyond this region toward the interior of the nodule there is a significant drop in $O_2$ concentration. A low $O_2$ level within the nodule is essential since the nitrogenase enzyme is very $O_2$ labile. However, the low $O_2$ level of the nodule interior creates a paradoxical situation for the rhizobial symbiont whose metabolism is totally dependent on $O_2$ (i.e., obligate aerobe). To circumvent the problem that rhizobia are obligate

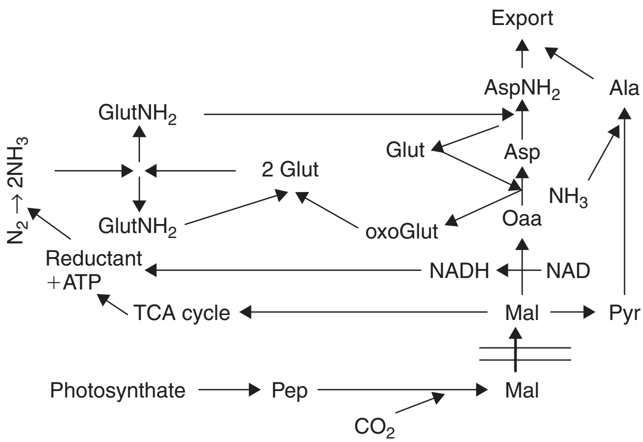



FIGURE 14.7 Simplified model of carbon and nitrogen metabolism in the bacteroid and the infected plant cell to illustrate the supply of C and energy from the plant and the assimilation of $NH_4^+$ produced by BNF.

aerobes and absolutely need $O_2$ to generate ATP, the infected cortical cells of the plant produce an $O_2$-carrying protein called leghemoglobin, which facilitates transfer of $O_2$ to the bacteroid. In addition, the bacteroid produces a specialized terminal cytochrome oxidase that enables it to respire under low-$O_2$ conditions.

As the rhizobia transform from free-living bacteria into bacteroids, their C metabolism is modified so that C is directed into the bacteroid to provide reductant and energy for BNF, and N assimilation is modified so that the plant benefits primarily from the BNF and not the rhizobia (Fig. 14.7). Malate and/or succinate are the carbon sources transported into the bacteroid and used in energy production and as C skeletons for bacteroid biosynthetic processes. Expression of plant genes involved with sucrose metabolism is enhanced in the rhizobia-infected plant cells. There is a severalfold increase in expression of sucrose synthase, which converts the photosynthate (sucrose) into precursors for the glycolytic pathway. Glycolysis breaks down the photosynthate to phosphoenolpyruvate, which can be processed to oxaloacetate and to malate by high levels of phosphoenolpyruvate carboxylase and malic dehydrogenase in the nodular plant cells.

The bacteroids catabolize malate and use the energy and reductant to produce ammonia via BNF. Until recently it was assumed that ammonia was directly transported from the bacteroid for assimilation into amino acids by plant nodule cells using GS, GOGAT, aspartate aminotransferase, and asparagine synthase. Waters *et al.* (1998) showed that *B. japonicum* bacteroids make alanine via reductive amination of pyruvate and transport it to the nodule cells. High bacteroid activities of malic enzyme (which converts malate to pyruvate) are consistent with malate having a second role of generating pyruvate for assimilation of $NH_3$ into alanine. Hence, ammonia and amino acids (alanine) may be the source(s) of N transferred

TABLE 14.10 Families and Genera of $N_2$-Fixing Plant–*Frankia* Actinorhizal Associations[a]

| Family | $N_2$-fixing genera |
|---|---|
| Betulaceae | *Alnus* |
| Casuarinaceae | *Casuarina* |
| Coriariaceae | *Coriaria* |
| Datiscaceae | *Datisca* |
| Elaeagnaceae | *Elaeagnus and Hippophae* |
| Myricaceae | *Myrica and Comptonia* |
| Rhamnaceae | *Ceanothus* |
| Rosaceae | *Cercocarpus and Purshia* |

[a]Reprinted from Paul and Clark (1996) by permission of the publisher.

to the plant cell for further processing. In legumes that form indeterminate nodules, N is transferred to growth sinks of the plant in the form of basic amino acids, asparagine and glutamine. In contrast, legumes that produce determinate nodules further convert fixed N from basic amino acids into compounds called ureides (allantoic acid, allantoin) that are transported to the growth sinks. Both types of molecules (basic amino acids and ureides) have extremely low C:N ratios and represent efficient N-transporting molecules.

## SYMBIOTIC ASSOCIATIONS BETWEEN ACTINORHIZAL PLANTS AND *FRANKIA*

Symbiotic associations are formed between the bacterial genus *Frankia* (gram positive, high G+C, filamentous bacterium) and many nonleguminous plant species widely distributed among eight different families and commonly referred to as actinorhizal species (Table 14.10). Well-known plant genera include *Alnus*, *Casuarina*, *Myrica*, *Hippophae*, *Elaeagnus*, *Ceanothus*, and *Purshia.* These plants are distributed worldwide and tend to be woody shrubs or trees that colonize N-limited landscapes that are usually in the primary stages of recovery from some disturbance (wild fire, landslide, flood, and logging). These plants colonize sites that range widely in latitude, elevation, and water and nutrient availability, and the amounts of $N_2$ fixed vary considerably ($<$10 to 300 kg N $ha^{-1}$ $year^{-1}$ (Table 14.5)).

In contrast to the first isolation of rhizobia over 100 years ago (1888), *Frankia* isolates were first obtained in culture in 1978 (Callaham *et al.*, 1978). *Frankia* has continued to be a notoriously difficult bacterium to isolate from nodules and to study in the laboratory; yet, considerable information has been obtained about it by using molecular biological methods that circumvent the need for obtaining pure cultures. Phylogenetic analyses of 16S rDNA and glutamine synthetase gene sequences of *Frankia* strains indicate that the genus belongs to three phylogenetically distinct clades or clusters (Fig. 14.8), Clawson *et al.*, 2004). Clade I is represented

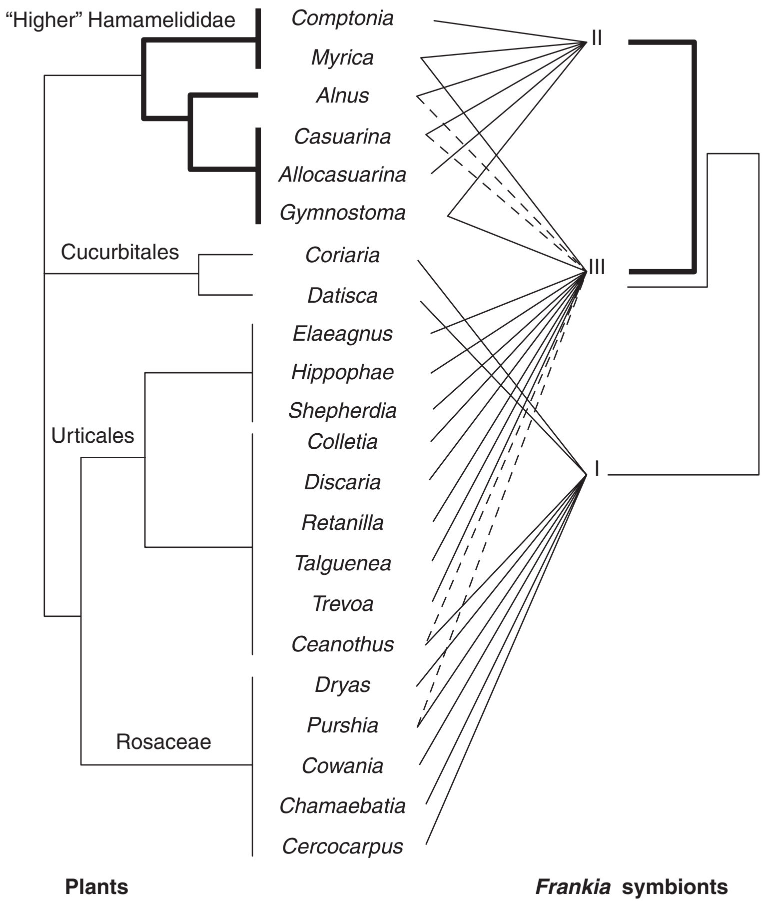



FIGURE 14.8 Relationships between actinorhizal host genera and three phylogenetically distinct clades of *Frankia* with emphasis on the mode of root infection that initiates nodule formation. On the left of the diagram, thick lines denote plant genera in which root infection occurs via root hairs, thin lines denote plant genera in which infection occurs via intercellular penetration of the root surface. On the right side of the diagram, solid lines indicate the presence of typical *Frankia* strains in nodules of a plant genus. Dashed lines indicate that *Frankia* clade III strains are rarely found in nodules on these plant genera. Reprinted from Clawson *et al.* (2004) by permission of the publisher; copyright (2004) Elsevier.

exclusively by microsymbionts that have been detected in nodules of a wide variety of hosts, but never obtained into culture. Clade II isolates are associated with a few genera of actinorhizal plants (e.g., *Casuarina*, *Alnus*, *Myrica*) that establish their symbioses through a root-hair infection process similar to that of legumes. Clade III strains tend to range widely across hosts that primarily practice the intercellular infection process.

There are many similarities between legumes and actinorhizals in the mode of plant infection, symbiosis establishment, and N assimilation, but there are also many differences. Whereas legume nodules represent stem-like organs, with a peripheral vascular system and nodule primordia that originate in the root cortex, actinorhizal nodules consist of modified lateral roots, possess a central vascular system, and arise from nodule primordia originating in the root pericycle. Recently, it was shown that the expression of some *nodulin* genes associated with BNF and N metabolism is more similar among legumes and intracellularly infected actinorhizal plant species than among the different phylogenetic groups of actinorhizal plants (Pawlowski and Bisseling, 1996). In *Frankia*, $N_2$ fixation occurs in swellings located at the tips of filaments referred to as vesicles. In the symbiotic state, vesicles are produced in large numbers, with vesicle shape and the degree of septation differing among different host plant species. For example, spherical–septated vesicles are found in *Alnus* and elongated–nonseptated vesicles in *Coriaria.* Interestingly, vesicles are not found in infected cells of *Casuarina* nodules.

Similar to the legume–rhizobia associations, some actinorhizal plants such as *Casuarina* and *Myrica* produce an $O_2$-transporting hemoglobin that correlates with the presence of an $O_2$ diffusion barrier surrounding infected cells. Most actinorhizals do not produce hemoglobin, however, and it is generally felt that the vesicle wall of the *Frankia* itself is the major diffusion barrier to $O_2$. In *Coraria*, infected cells are surrounded by a suberized periderm layer whose thickness varies with $O_2$ concentration.

## BIOTECHNOLOGY OF BNF

With the world's population projected to increase to 8.3 billion by 2025, and the accompanying demand for plant nutrient N, there will continue to be a need to increase the contribution of BNF to food and fiber production. As mentioned earlier in this chapter, rates of BNF under field conditions vary widely, and there are many environmental/agronomic factors generally associated with plant nutrition, water availability, and plant disease that hinder $N_2$-fixing species from reaching their yield potential under field conditions. We should not overlook the fact that some undesirable attributes of $N_2$-fixing species, such as toxin production, along with ethnical/historical biases, disfavor their use as major food and fiber sources in some parts of the world. The Leguminosae is an enormous plant family distributed worldwide, with 16,000 to 19,000 species in about 750 genera (Allen and Allen, 1981). Despite this wealth of diversity, very few legumes have been successfully domesticated and used worldwide by humans for agricultural purposes. For many years attempts to expand the agricultural use of leguminous species beyond their regions of origin had a checkered history because the appropriate rhizobia were not always present in the soil. As a consequence, it has been standard practice for many years to inoculate legume seed with appropriate rhizobia before planting a legume species in regions where (a) it has not been grown previously, (b) the

native flora of the region did not contain legumes that were close relatives of the crop, and (c) an interval of several years exists between the use of a legume in the rotation. Another example of a situation in which inoculation is recommended includes replacement of an older established cultivar of a legume species with a new disease-resistant variety. The new variety might not form a highly effective symbiotic association with native rhizobial strains that were effective on the older cultivar. During the 20th century, agronomists and soil microbiologists recognized that many environments in which crop production and soil quality could benefit tremendously from BNF contain conditions hostile for survival of rhizobia. As a result, inoculant-quality rhizobial strains are usually prescreened for their ability to tolerate low pH, high aluminum, high temperature, and desiccation and to be genetically stable. Nonetheless, despite our best efforts, we have not yet been able to overcome the facts that many soils contain indigenous or naturalized populations of rhizobia that are suboptimally effective at BNF on agronomically important legumes and that it is extremely difficult to displace them with superior $N_2$-fixing strains. As scientists make more progress in elucidating the molecular signals needed for nodulation by rhizobia and infection by mycorrhizal fungi, and with the genomic sequences of more bacteria and plants becoming available, it is not outside the realm of possibility that we will learn how to develop other symbiotic/endophytic associations between plants and $N_2$-fixing bacteria. The example of sugarcane and *Glu. diazotrophicus* gives hope for a future that includes $N_2$-fixing cereals and other food and fiber crops.

## ACKNOWLEDGMENTS

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# 15

# Soil Biogeochemical Cycling of Inorganic Nutrients and Metals

Alain F. Plante



## INTRODUCTION

Soil microorganisms have a profound effect on the transformations involved in a large number of biogeochemical cycles other than C and N, such as the macronutrients phosphorus (P) and sulfur (S), and various micronutrients and environmental pollutants. A conceptual model for the cycling of a generic nutrient or metal element (Fig. 15.1) illustrates the continuous flow of energy, water, nutrients, and other materials across the ecosystem's boundaries (from Likens and Bormann, 1999). Meteorologic transfers consist of windblown particulate matter, dissolved substances in precipitation, and gases. Geologic fluxes include soluble and particulate matter transported by surface and subsurface water flow and the mass movement of mineral materials during events such as erosion, landslides, or lava flows.

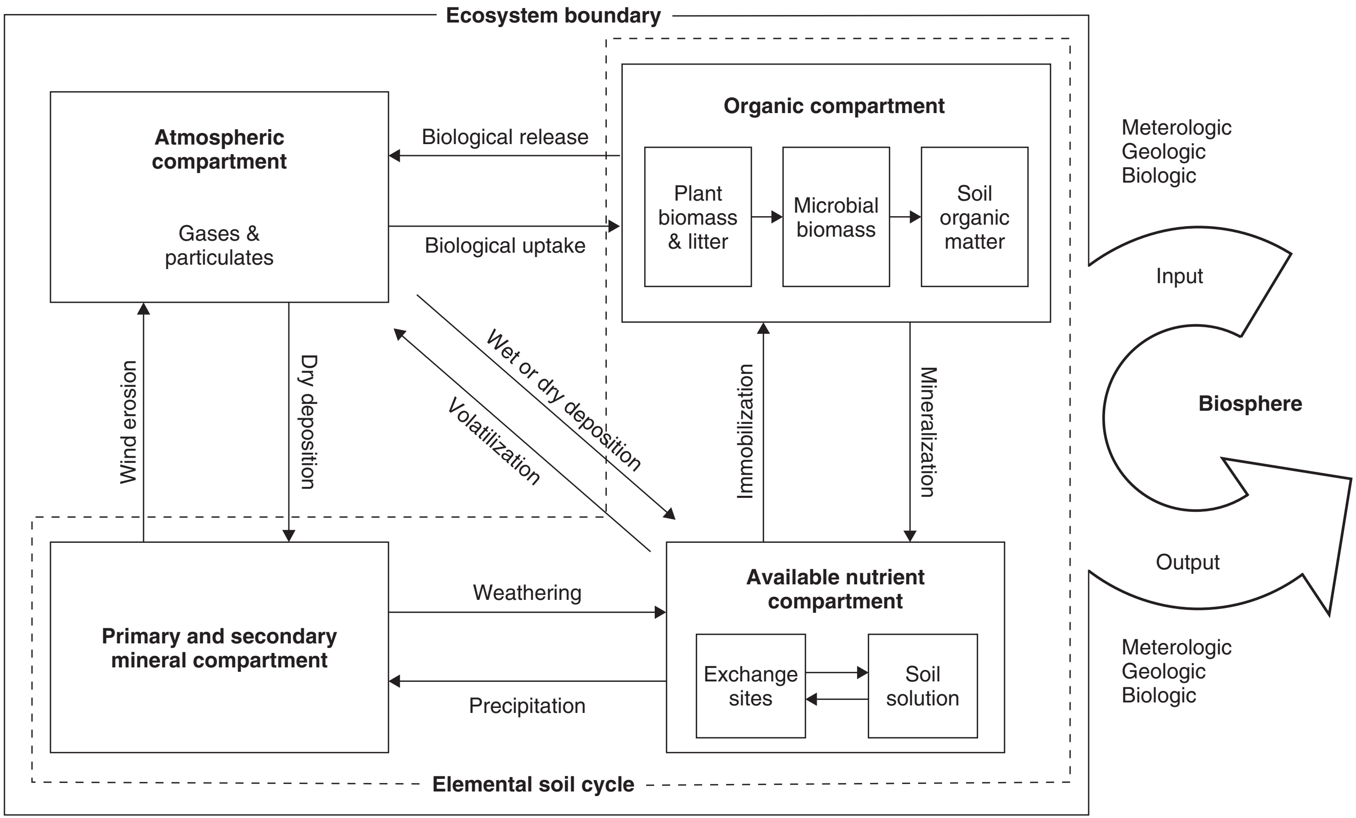



FIGURE 15.1 A conceptual representation of a generic elemental biogeochemistry cycle (with permission from Likens and Bormann, 1999).

Biologic fluxes result when chemicals or energy gathered by organisms in one ecosystem are deposited in another (e.g., entrainment of litter material from above the soil surface to subsurface layers). The nutrient or metal element may occur in four compartments within the soil ecosystem: (1) atmosphere, (2) living and dead organic matter, (3) available nutrients, and (4) primary and secondary minerals. Microbially mediated reactions that transform these elements include: (1) mineralization and immobilization, reactions that transform the element from organic to inorganic and inorganic to organic forms, respectively; (2) reduction and oxidation, reactions that involve the transfer of electrons; (3) solubilization, reactions by which relatively insoluble materials are rendered soluble and therefore available to plants or microorganisms; (4) volatilization, reactions that transform an element to a volatile or gaseous form; and (5) detoxification, reactions that reduce the toxicity of an element to the microorganism in question. The last group of reactions includes redox reactions as well as alkylation reactions.

The ability of soil microorganisms to oxidize or reduce several elements has likely developed in response to changing environments over the course of evolution and is now evident in gradient environments such as soil where $O_2$ is more or less available due to water- versus air-filled porosity (Fig. 15.2). In aerobic environments, stoichiometry may integrate other element cycles, but energy generation is dominated by a union between the C and the O cycles. However, when the redox potential ($E_h$) decreases, a number of other element cycles become more closely integrated as alternative electron acceptors (e.g., $Fe^{3+}$, $Mn^{4+}$, $NO_3^-$, $SO_4^{2-}$) are utilized by various groups of organisms in microbially mediated oxidation and reduction reactions. An example of element cycle integration is the autotrophic facultative anaerobic bacterium *Thiobacillus denitrificans*, which is capable of oxidizing sulfide to elemental S using nitrate as its electron acceptor and carbon dioxide as its sole C source under anoxic conditions. The organism can accumulate S extracellularly and converts the nitrate to nitrogen gas. Other examples of element cycle integrations are illustrated under Environmental Significance of P, S, and Metal Biogeochemistry, in which several examples of the unification of Fe and S cycles during the processes of acid mine drainage formation and corrosion are given. Our improved understanding of the role of microorganisms in the biogeochemistry of many elements, through the application of modern molecular and genetic tools, will certainly continue to contribute to the development of strategies to protect the environment from contamination and to extract and recycle valuable resources in a sustainable manner.

# PHOSPHORUS

## THE SOIL PHOSPHORUS CYCLE

Nitrogen and phosphorus are the elements that most often limit biological productivity. While the major source of N is atmospherically derived, P is primarily

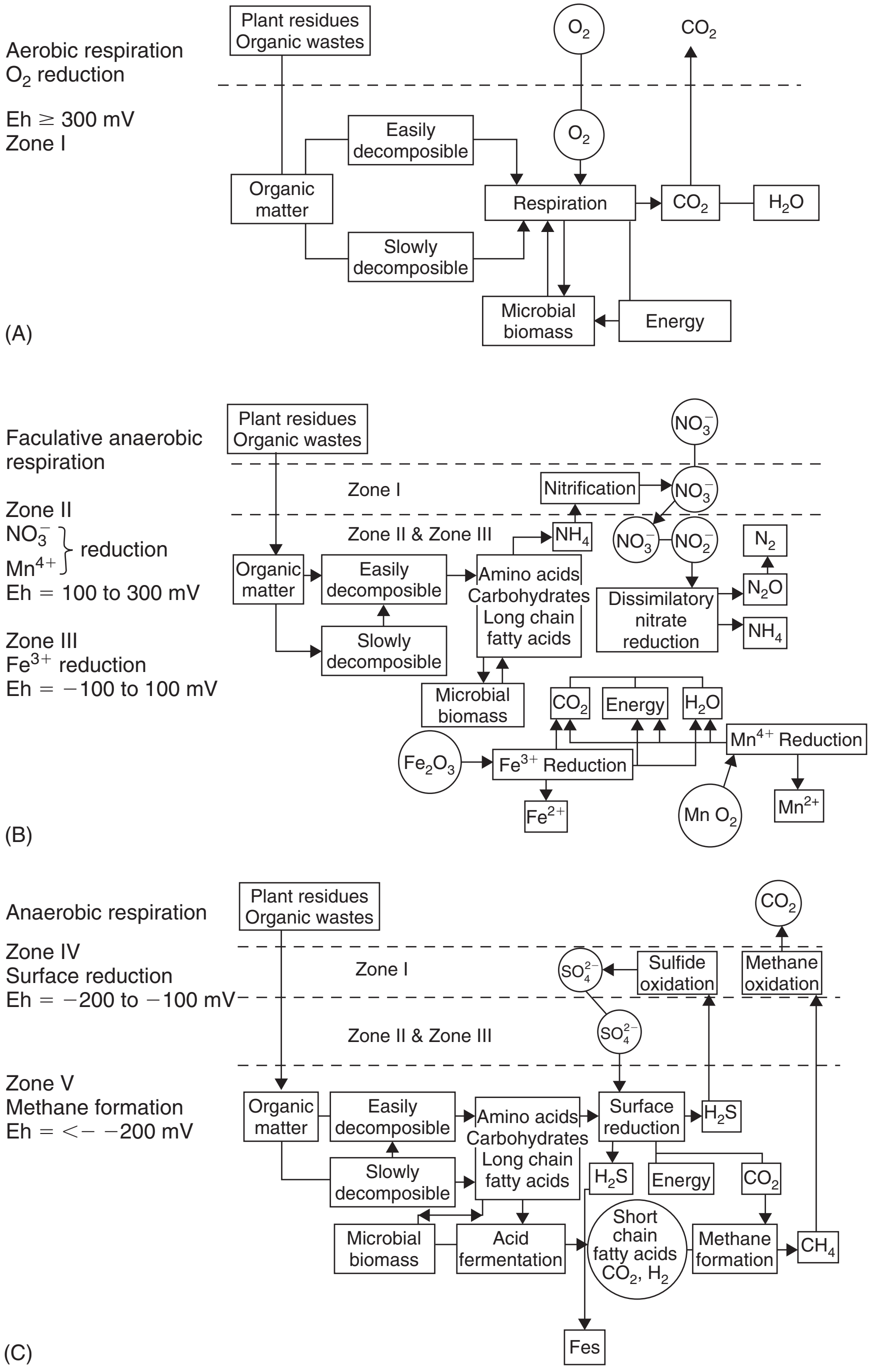



FIGURE 15.2 Pathways of organic matter decomposition during (A) aerobic respiration, (B) facultative anaerobic respiration, and (C) anaerobic pathways (with permission from Reddy *et al.*, 1986).

rock derived, which severely restricts its input rate and makes it depletable (Walker and Syers, 1976). In highly weathered soils, where P is no longer supplied from the parent material, dust deposition may represent the majority of P inputs (Vitousek, 2004). The soil P cycle has been represented in several forms, depending on the particular emphasis used by the author (e.g., soil solution P, soil–plant relations, or microbially mediated P transformations). In Fig. 15.3, soil solution P is placed in the central position, and the P cycle is divided into two subcycles: a biological one in which pools are defined in terms of biological constituents or stability and transfers are primarily microbially mediated, and a geochemical subcycle in which pools are defined in terms of chemical or mineralogical composition and transfers are primarily abiotic. While soil P is not particularly mobile, some losses from soil in dissolved organic form have been observed in highly weathered soils and were found to be of a magnitude similar to that of inputs (Vitousek, 2004). Unlike C and N, phosphorus does not show large biologically mediated fluxes to and from the atmosphere, nor does it serve as a primary energy source for microbial oxidations. Nevertheless, soil organisms are intimately involved in the cycling of soil P. They participate in the solubilization of inorganic P and in the mineralization of organic P. They are also important in the immobilization of available soil P.

## NATURE AND FORMS OF PHOSPHORUS IN SOIL

The largest global reservoir of P is the ocean sediment pool (Table 15.1), which represents a small but steady sink for terrestrial P released by the weathering of minerals or released by the biota. Phosphorus occurs mainly in inorganic phosphates and in organic phosphate derivatives. The primary mineral form of P is apatite, with the basic formula $M_{10}(PO_4)_6X_2$. Commonly, the mineral (M) is Ca and less often Al or Fe. The anion (X) is $F^-$, $Cl^-$, $OH^-$, or $CO_3^{2-}$, thus creating fluor-, chloro-, hydroxyl-, or carbonate apatites. Diverse substitutions and combinations of M and X result in some 200 forms of inorganic P. Total P concentrations in soils range from 35 to 5300 $\mu g\ g^{-1}$ with an average of 800 $\mu g\ g^{-1}$ (Bowen, 1979; Table 15.2). Highly weathered soils may have very little P, while weakly weathered soils and those soils formed from alkaline parent materials may have high P contents. The chemical weathering of apatite results in the release of orthophosphate ($H_2PO_4^-$ is the dominant species at pH $< 7.2$, while $HPO_4^{2-}$ dominates at pH $> 7.2$).

Very little orthophosphate is present in soil solution at any one time. The concentration of P in the soil solution rarely exceeds 0.1 to 1 $\mu g\ g^{-1}$, representing $<1\%$ of the total P. Solubility of P is complicated by common ion–ion association and pH effects and the amount of P adsorbed on clay mineral surfaces. Soluble P rapidly precipitates as iron and aluminum phosphates in acid soils or as calcium phosphates in alkaline soils or is adsorbed to iron and aluminum oxides or clay mineral surfaces. The optimum availability of orthophosphate occurs at a soil pH of approximately 6.5, at which aluminum and calcium precipitation is at a minimum.

The hundreds of structural forms of inorganic P in nature necessitate their discussion on the basis of extractability and availability to plants and microorganisms.

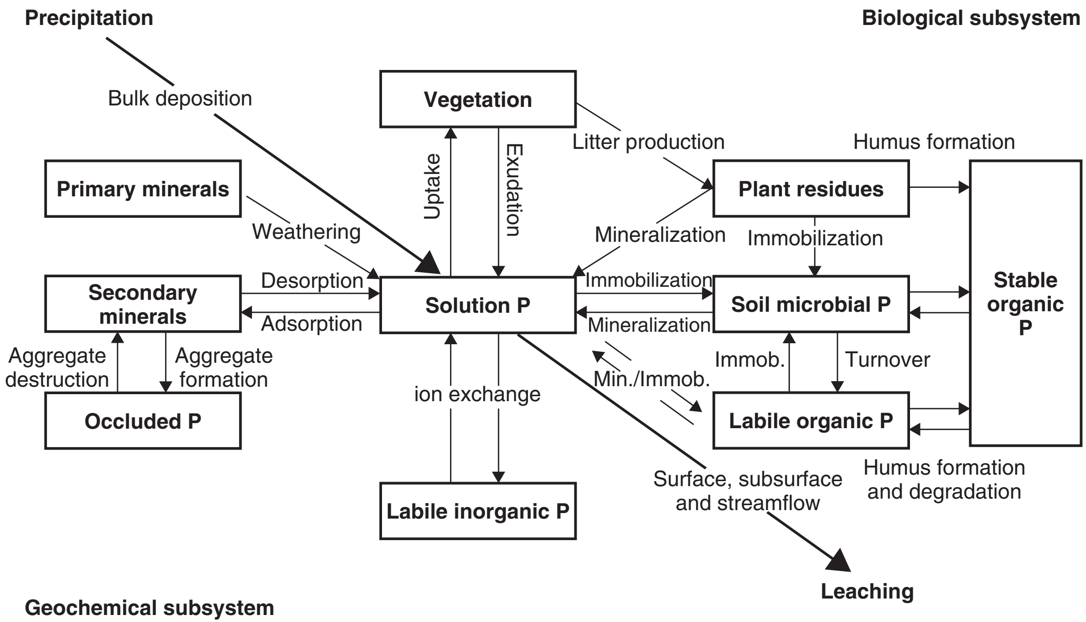



FIGURE 15.3 Schematic representation of the phosphorus cycle consisting of a biological subcycle in the upper right and a geochemical subcycle in the lower left (with permission from Walbridge, 1991).

**TABLE 15.1** Major Reservoirs of Phosphorus in the Earth[a]

| Reservoir | Total phosphorus ($\times 10^{15}$ g) |
|---|---|
| Atmosphere (particulates) | 0.000028 |
| Land | |
| Soil | 96–160 |
| Mineable rock (high apatite) | 19 |
| Biota | 2.6 |
| Fresh water (dissolved) | 0.090 |
| Ocean | |
| Sediments | 840,000 |
| Dissolved (organic) | 80 |
| Detritus (particulates) | 0.65 |
| Biota | 0.050–0.12 |

[a] With permission from Bolin *et al.* (1983).

**TABLE 15.2** Total and Organic Phosphorus Concentrations in the Surface Layer (0–10 cm) of Some North American Grassland Soils

| Site | Total P ($\mu g\ g^{-1}$) | Organic P ($\mu g\ g^{-1}$) |
|---|---|---|
| Cottonwood, South Dakota, USA | 554 | 310 |
| Bridger, Montana, USA | 1234 | 675 |
| Osage, Kansas, USA | 251 | 227 |
| Ale, Washington, USA | 748 | 29 |
| Jornado, New Mexico, USA | 445 | 37 |
| Pantex, Texas, USA | 94 | 239 |
| Bison, Manitoba, Canada | 835 | 582 |
| Pawnee, Colorado, USA | 345 | 131 |

When P is added to soil as a soluble salt (fertilizer), it becomes fixed or bound to the extent that very little of the added P is reextractable with water. Hedley *et al.* (1982) developed a sequential extraction procedure to separate soil P into inorganic and organic pools based on their availability to plants. The major fraction of P is not extractable by dilute acids or bicarbonate solution. This portion of the retained P is commonly designated as fixed P. The portion that is extractable by dilute acid or bicarbonate is designated as available P; it is considered to be the amount of soil P available for uptake by living organisms. Available P measured using this approach is partly inorganic and partly organic. That portion of the total P in soil that is resin extractable is designated as exchangeable P, defined as that fraction of the soil P that can enter solution by isoionic exchange during a given time span.

Phosphorus in organic molecules constitutes 30–50% of the total P in most soils, ranging from as low as 5% to as high as 95%. Despite the importance of soil organic P, its chemical nature has not been fully characterized. This is due in part to analytical limitations, as there are no direct methods to characterize soil organic

P. Even solid-state phosphorus-31 nuclear magnetic resonance ($^{31}P$ NMR) spectroscopy cannot detect organic P in soil because of poor sensitivity and interference from paramagnetic ions. Organic P must therefore be extracted from soil before it can be quantified and identified (see Turner *et al.*, 2005). Organic P occurs in soil principally as phytates or related forms, nucleic acids and their derivatives, and phospholipids (Fig. 15.4). Phytin, primarily polymeric inositol hexaphosphate, is synthesized by plants and accounts for roughly 40% of the organic P found in soil. Some of the polymers are believed to be of microbial origin. Some yeasts synthesize phosphorylated polymers of mannose and the cell walls of $G^+$ bacteria contain both techoic acid, a polymer of ribitol phosphate and glycerophosphate, and a 6-phosphate muramic acid. Lower weight inositol ring compounds carrying one to five atoms of P also occur in soil and probably represent degradation products of inositol hexaphosphate.

Constituent parts of nucleic acid molecules are identifiable in hydrolysates of soil extracts. These include cytosine, adenine, guanine, uracil, hypoxanthine, and xanthine. The last two are decomposition products of guanine and adenine. Of the total organic P in soil, only about 1% can be identified as nucleic acids or their derivatives. The susceptibility of nucleic acids to decomposition, together with a lack of incorporation into stable organic matter, is believed to be responsible for their low level of occurrence in soil. Extracellular DNA sorbed to soil mineral surfaces persists from 100 to 1000 times as long as free DNA. This mineral-associated DNA is considered to provide for natural genetic transformation of bacteria.

Organic P in alcohol and ether extracts of soil is indicative of the presence of phospholipids. Choline has been identified; it is one of the products of hydrolysis of lecithin. Most of the glycerophosphate found in soil is believed to be of lipid origin. Phospholipid P accounts for a similarly low fraction of the soil organic P as does nucleic acid P. Even smaller amounts of sugar phosphates are found. These are easily decomposable and therefore, together with nucleic acid and phospholipid P, cannot be considered as contributing significantly to the estimates of 350 to 2000 years as the mean residence time for total organic P in a prairie soil.

While the sizes of the organic P pools in soil generally occur in the order inositol phosphate > polymer organic phosphate > nucleic acid P > phospholipid P, the concentrations of these pools within the soil biota occur in the reverse sequence. Next to N, P is the most abundant nutrient contained in the microbial biomass. Brookes *et al.* (1982) developed a method to measure P within the soil microbial biomass using chloroform ($CHCl_3$) fumigation followed by extraction with $NaHCO_3$ and relating the measured P to the P extracted from an aliquot not treated with $CHCl_3$. Work with soils having pH of 6.2 to 8.2 showed a recovery ($K_p$) of 40% of added $^{32}P$-labeled cells, suggesting a calculation of microbial P as

$$\text{microbial P} = (\text{CHCl}_3\ \text{P} - \text{control P})/K_p,$$

where $K_p = 0.4$. Microbial biomass P concentrations of 5 to 75 $\mu g\ P\ g^{-1}$ soil were reported, representing from 2 to 5% of the total organic phosphorus in cultivated soil and up to 20% in grassland and forested soils.

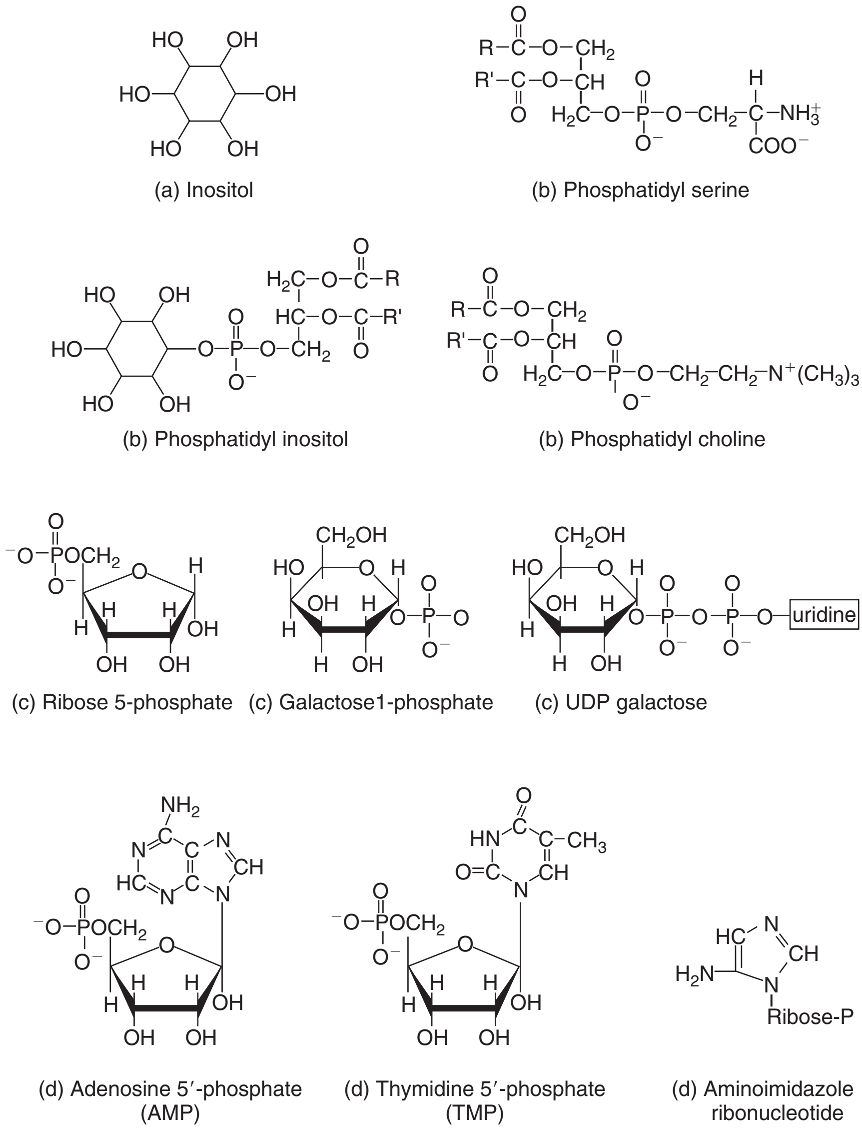



FIGURE 15.4 Organic phosphorus compounds. (a) Inositol. If given six phosphorus substitutions on the ring (C–P linkages), it becomes inositol hexaphosphate. Fewer substitutions yield 1-, 2-, 3-, 4-, or 5-phosphatidyl inositol phosphates. (b) Phosphoglycerides (C–O–P linkages), shown linked to serine. (c) Phosphate sugars. (d) Nucleic acid components.

## BIOLOGICAL IMPORTANCE OF PHOSPHORUS

Phosphorus is an element essential to life. It plays both structural and functional roles in virtually all organisms and is found in cell components such as phospholipids, nucleic acids, and DNA as described above. Phosphorus, through

**TABLE 15.3** Carbon:Nitrogen:Phosphorus Ratios in Organisms and Soil Organic Matter

| | C | N | P |
|---|---|---|---|
| Marine algae | 106 | 16 | 1 |
| Soil bacteria | 31 | 5 | 1 |
| Grassland soil | | | |
| Native | 191 | 6 | 1 |
| Cultivated, fertilized | 119 | 9 | 1 |
| Weakly weathered soil | 80 | 5 | 1 |
| Strongly weathered soil | 200 | 10 | 1 |

the phosphate anhydride bond (Fig. 15.4d), plays an important role in storing and transferring biochemically useful energy. A free energy change ($\Delta G^0$) of $-7.3$ kcal $mol^{-1}$ ($-30.6$ kJ $mol^{-1}$) of adenosine 5′-triphosphate (ATP) is associated with the hydrolysis of its terminal anhydride bond, yielding adenosine 5′-diphosphate and a phosphate group (see Chap. 9). Unlike many other anhydrides, phosphate anhydrides such as ATP are unusually resistant to hydrolysis in the aqueous environment (Westheimer, 1987). At neutral pH and physiological temperature, hydrolysis proceeds at an optimal rate only in the presence of appropriate enzymes (e.g., ATPase). The relative resistance of phosphate anhydride bonds to hydrolysis is attributable to the negative charges on the phosphates at neutral pH and is the probably reason ATP was selected in the evolution of life as a universal transfer agent of chemical energy in biological systems (Westheimer, 1987).

There is good evidence that P is the dominant element controlling C and N immobilization in biological systems. In a classic paper, Redfield (1958) hypothesized that P controls the C, N, and S cycles of marine systems. He noted that the oceanic C:N:P ratio paralleled that of the plankton and believed the following general relationship occurs:

$$106CO_2 + 16NO_3^- + 122H_2O + 18H^+ \xrightarrow{\text{solar energy, trace elements}} C_{106}H_{263}O_{110}N_{16}P_1 + 138O_2.$$

The C:N:P ratios for a number of terrestrial situations are shown in Table 15.3. The aquatic algae and soil bacteria have similar C:N ratios of approximately 6:1. The algae have lower C:P ratios than the bacteria, but fall within the range of C:P found in soil organic matter.

## MICROBIAL TRANSFORMATIONS OF PHOSPHORUS

### Mineralization

Organically bound P is not directly available to organisms because it cannot be absorbed into cells in this form. For cellular uptake to occur, P must first be released from the organic molecule through mineralization. The final stage in the conversion of organically bound P to inorganic phosphate occurs through the action of phosphatase

enzymes. The phosphatase group of enzymes includes phytase enzymes that catalyze the release of phosphate from phytin and nuclease enzymes that liberate phosphate from nucleic acids. These enzymes are produced by up to 70–80% of the microbial population, including bacteria such as *Bacillus megaterium*, *B. subtilis*, *Serratia* spp., *Proteus* spp., *Arthrobacter* spp., and *Streptomyces* spp. and fungi such as *Aspergillus* spp., *Penicillium* spp., *Rhizopus* spp., and *Cunninghamella* spp. Once P is mineralized, it can be taken up by plants, immobilized by the microbial biomass, precipitated in inorganic complexes, or sorbed to mineral surfaces.

### Immobilization

Soil microorganisms can cause fixation or immobilization of P, either by promoting the formation of inorganic precipitates or by assimilation into organic cell constituents or intracellular polyphosphate granules. In soils and freshwater sediments, cellular immobilization is important, though fixation of P by $Ca^{2+}$, $Al^{3+}$, or $Fe^{3+}$ has been observed. In some marine sediments, where phosphorite minerals occur, the precipitation mechanism is more important. Microorganisms are indirectly involved in phosphorite precipitation by making reactive phosphate available, by making reactive calcium available, or by creating or maintaining the environmental conditions that favor phosphate precipitation.

The extent of immobilization of P is affected by the C:P ratio of the organic materials being decomposed and the amount of available P in solution. If insufficient P is available in the substrate for assimilation of the substrate C, inorganic P from the soil solution will be used and net immobilization occurs. Generally, a C:P $< 200$ will result in net mineralization, a C:P $> 300$ results in net immobilization, and C:P ratios between 200 and 300 result in little net change in soluble P concentrations.

### Oxidation and Reduction

A number of soil bacteria and fungi have been shown to be capable of oxidizing reduced phosphorus compounds (e.g., phosphite, hypophosphite) either aerobically (Adams and Conrad, 1953) or anaerobically (Foster *et al.*, 1978). The biochemical pathway for such a microbially mediated reaction has been characterized molecularly and genetically, providing some evidence for a previously underappreciated microbial redox cycle for P. The relatively high solubility in water of phosphites, hypophosphites, and phosphonates suggests they may have been important precursors of biochemical P compounds, but the fact that there have been only trace quantities of phosphite and hypophosphite detected in the current environment suggests that the existence of microbial pathways of P oxidation might represent an ancient evolutionary property (Foster *et al.*, 1978; Schink and Friedrich, 2000).

There is increasing appreciation in the literature for the presence of reduced forms of P such as phosphine ($PH_3$), phosphites, and organic phosphonates, which provide a small gaseous link to the P cycle (Glindemann *et al.*, 2005). Microbially mediated reduction of phosphate remains a controversial topic in the literature (see Morton and Edwards, 2005; Roels and Verstraete, 2001). The controversy is fueled by thermodynamic calculations that show that the reduction of phosphate is energetically unfavorable. However, this does not imply that reduced P compounds

cannot be formed biogenically because other linked mechanisms exist in nature by which energetically unfavorable reactions, such as $N_2$ fixation, can occur.

**Solubilization**

The low solubility of P in soils makes it one of the major nutrients limiting plant growth. Frequent applications of soluble forms of P are needed, more than really necessary, because only a fraction is used by plants while the rest rapidly forms insoluble complexes. Traditional P fertilizer production is based on chemical processing of insoluble mineral phosphate ore, which is expensive and environmentally undesirable. In areas where commercially produced P fertilizer is too costly, the microbial solubilization of phosphate rock is seen as a viable alternative (see Whitelaw, 2000). In India, for example, there is an estimated 40 million tons of P-containing rock deposits that could provide a cheap source of P fertilizer. One strategy currently used is to mix rock phosphates with various plant residues in a composting mixture. In some cases, the compost is enriched with known P-solubilizing bacteria. The development of agricultural inoculants has been difficult and knowledge of the genetics of phosphate solubilization is still sparse (Rodriguez *et al*., 2006).

Phosphate-solubilizing microorganisms are suspected to convert the insoluble rock phosphates into soluble forms through the processes of acidification, chelation, and exchange reactions, but uncertainty remains concerning the detailed mechanisms, which may be organism-dependent. Carbonic acid and $HCO_3^-$ derived from respiratory $CO_2$ are of prime importance in the weathering of soil minerals, but there is poor correlation between $CO_2$ levels and dissolution of apatite. Illmer and Schinner (1995) showed that *Aspergillus niger* produced citrate, oxalate, and gluconate and suggested that organic acid production may be an important mechanism for solubilizing aluminum phosphates, but not the only effective mechanism. They found that other organisms, such as *Penicillium aurantiongriseum* and *Pseudomonas* sp. (*PI*18/89), were effective at solubilizing aluminum or calcium phosphates without producing organic acids. Proton release associated with respiration or ammonium assimilation was proposed as the mechanism responsible. Organic acids produced in the rhizosphere by plant roots and associated microorganisms may act as chelating agents. These organic chelates form complexes with Ca, Fe, or Al, thereby releasing the phosphates to solution. It is currently impossible to select any given mechanism from the alternatives, and some researchers have questioned whether sufficient acidity or chelating agents can be generated microbially to appreciably affect P solubility.

## SULFUR

### THE SOIL SULFUR CYCLE

The terrestrial S cycle involves significant interactions between the pedosphere, the hydrosphere, the biosphere, and the atmosphere. The S cycle (Fig. 15.5) is

similar to the soil N cycle, and unlike P, these elements undergo chemical and microbially mediated transformations leading to volatilization. Not only is the biosphere a repository for highly mobile forms of S, but several key reactions of the cycle are accelerated by, and sometimes completely controlled by, microbiological activity. The soil microbial biomass acts as the driving force behind mineralization–immobilization and oxidation–reduction transformations. The primary input of S to soil occurs during the weathering of soils, which releases sulfate into the available pool. Other inputs include plant residue inputs, S fertilizer, pesticides, and irrigation water. Atmospheric deposition can be a significant input of S in areas affected by atmospheric pollution due to combustion of fossil fuels. Losses of S from the soil system include plant uptake and harvesting of residues, long-term fixation into minerals, leaching of soluble sulfate, and gaseous losses of volatile forms of S.

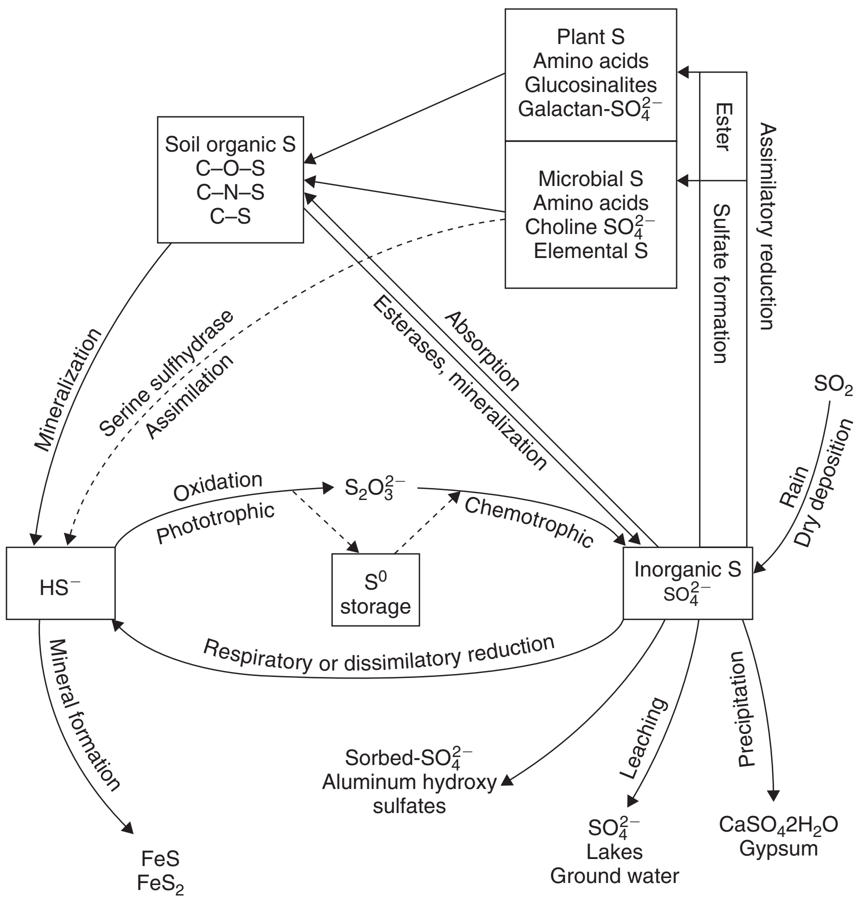



FIGURE 15.5 Sulfur (S) transformations in nature. Elemental sulfur is shown as a storage product, and the possibility of sulfate sorption in certain soils is included.

TABLE 15.4 Major Reservoirs of Sulfur in the Earth[a]

| Reservoir | Total sulfur ($\times 10^{15}$ g) |
|---|---|
| Atmosphere | 0.0048 |
| Biosphere | 0.76 |
| Hydrosphere | 1,300,000 |
| Oceans | 1,300,000 |
| Marine organisms | 0.024 |
| Fresh waters | 3.0 |
| Ice | 6.0 |
| Pedosphere | 260 |
| Soil organic matter | 1.1 |
| Lithosphere | 24,300,000 |
| Continental, sedimentary | 5,200,000 |
| Continental, granite | 7,800,000 |
| Continental, basalt | 8,800,000 |
| Oceanic | 2,500,000 |

[a] With permission from Bolin *et al.* (1983).

## NATURE AND FORMS OF SULFUR IN SOIL

The largest global reservoir of S is the lithosphere (Table 15.4). The atmospheric content of S represents a relatively small pool, but one that has increased significantly in recent times due to the burning of fossil fuels. Atmospheric emissions of S peaked at approximately 70 million metric tons (70 Tg) on an annual basis in the mid- to late-1980s. The result is acid rain, containing $SO_4^{2-}$ and $NO_3^-$, which is acidifying surface waters and soils. These emissions have declined in recent decades where pollution abatement has been implemented. Areas with minimal atmospheric pollution receive approximately 1 kg S $ha^{-1}$ $year^{-1}$, while areas downwind from heavily industrialized areas can receive as much as 100 kg S $ha^{-1}$ $year^{-1}$. Many soils near cities receive more S from dry deposition through particulates than wet deposition from S dissolved in rainfall. Gases such as hydrogen sulfide ($H_2S$), carbon disulfide ($CS_2$), carbonyl sulfide (COS), methyl mercaptan ($CH_3SH$), dimethyl sulfide ($[CH_3]_2S$), and dimethyl disulfide ($[CH_3]_2S_2$) also enter the atmosphere through microbial transformations of both organic and inorganic S. Atmospheric S in aerosol form is most important to global climate change because of its impact on haze formation and changing the earth's reflectance to sunlight.

Most agricultural soils contain S in the range 20–2000 μg S $g^{-1}$ (Table 15.5). Many volcanic ash, organic, and tidal marsh soils contain 3000 or more μg S $g^{-1}$, and some desert soils have a total S content in excess of 10,000 μg S $g^{-1}$. In soils other than aridisols, the inorganic S fraction is typically small compared to the organic fraction (Table 15.5). Inorganic S exists in a number of oxidation states, ranging from +6 in $SO_4^{2-}$ to −2 in $H_2S$ and its derivatives (Table 15.6). More

**TABLE 15.5** Amounts and Distribution of Sulfur in Some World Soils

| Location | Type of soil | Total sulfur ($\mu g\ g^{-1}$) | Total sulfur ($\times 10^6$ g): Inorganic, reducible | Organic: C-bonded | Organic: Ester sulfate |
|---|---|---|---|---|---|
| Saskatchewan, Canada | Agricultural | 88–760 | 0.5–13 | 29–59 | 41–71 |
| British Columbia, Canada | Grassland | 286–928 | ND | 31–61 | 39–69 |
| | Forest | 162–2328 | ND | 20–47 | 53–80 |
| | Organic | 1,122–30,430 | ND | 28–75 | 25–72 |
| | Agricultural | 214–438 | 2 | 18–45 | 55–82 |
| Iowa, USA | Agricultural | 57–618 | 2–8 | 43–60 | 7–18 |
| Carolinas, USA | Tidal marsh | 3,000–35,000 | — | — | — |
| Hawaii, USA | Volcanic ash | 180–2200 | 6–50 | 50–94 | 50–94 |
| Eastern Australia | Agricultural | 38–545 | 4–13 | 10–70 | 24–76 |
| Nigeria | Agricultural | 25–177 | 4–20 | 80–96 | 80–96 |
| Brazil | Agricultural | 43–398 | 5–23 | 20–65 | 24–59 |

**TABLE 15.6** Oxidation States of Microbiologically Important Forms of Sulfur in Soil

| Compound | Formula | Oxidation state(s) of sulfur |
|---|---|---|
| Sulfate | $SO_4^{2-}$ | +6 |
| Pentathionate | $S_5O_6^{2-}$ | –2, +6 |
| Tetrathionate | $S_4O_6^{2-}$ | –2, +6 |
| Trithionate | $S_3O_6^{2-}$ | –2, +6 |
| Dithionate | $S_2O_6^{2-}$ | +6 |
| Thiosulfate | $S_2O_3^{2-}$ | –1, +5 |
| Sulfite | $SO_3^{2-}$ | +4 |
| Hyposulfite (dithionite) | $S_2O_4^{2-}$ | +3 |
| Sulfur | $S_8$ | 0 |
| Polysulfide | $S_n^{2-}$ | –2, 0 |
| Sulfide | $S^{2-}$ | –2 |

than 2000 S-bearing minerals with S contents ranging from 7 to 53% make S the 13th most abundant element in the earth's crust. Weathering during soil formation occurs very slowly, and primary mineral S is seldom a significant source of plant-available S. Inorganic S exists primarily as $SO_4^{2-}$ in calcareous soils of semiarid and arid regions, in volcanic ash, and in recently reclaimed tidal marsh areas. It may be present as gypsum ($CaSO_4$), as a basic aluminum sulfate, or as a contaminant of $CaCO_3$ in concentrations reaching as high as 3000 $\mu g\ g^{-1}$.

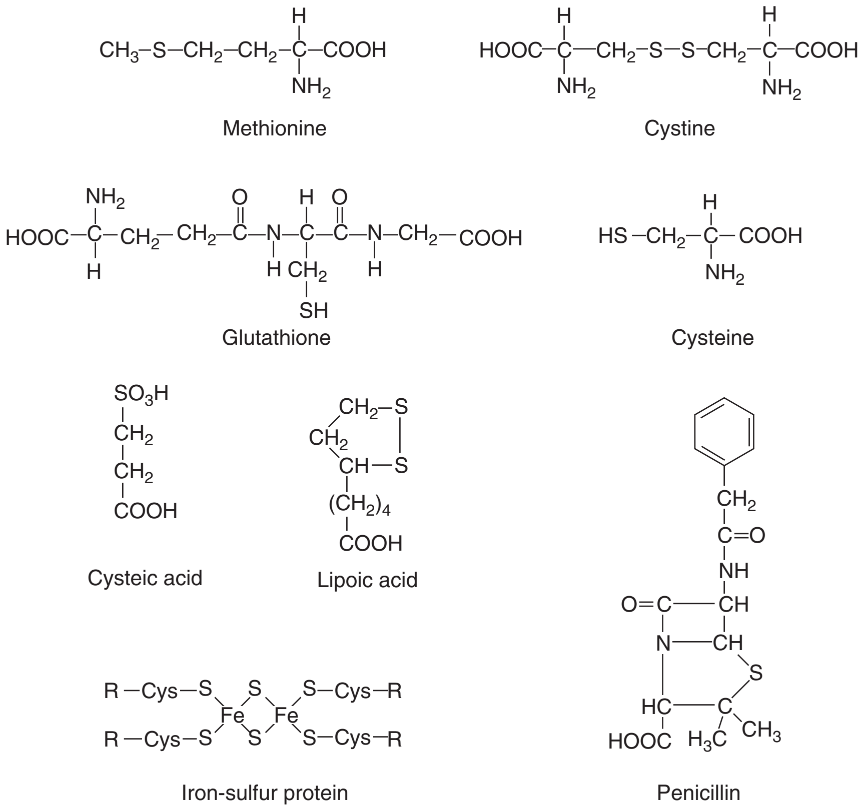



FIGURE 15.6 Forms of carbon-bonded sulfur.

Organic forms of S represent up to 90% of the total soil S. These forms can be classified into two major types. C-bonded S constitutes up to 30% of the organic S and occurs in amino acids such as cysteine, cystine, and methionine; Fe–S proteins called ferrodoxodins; polypeptides; heterocyclic compounds such as biotin and thiamine, sulfinates, sulfones, sulfonates, and sulfoxides (Fig. 15.6). Sulfate esters are the second group and constitute 30–75% of the organic S in soil. These include sulfate esters (C–O–S), sulfamates (C–N–S), and sulfated thioglycosides (N–O–S) (Fig. 15.7). The measurement of this form involves reduction with hydroioidic acid (HI); thus it is often referred to as HI-reducible S. This is considered to be the more biologically active or labile form in soil.

Soil microbial biomass S represents 2–3% of the total organic soil S. Microbial S is measured by lysing the cells with chloroform and measuring the S released to a $CaCl_2$ or $NaHCO_3$ extractant (Saggar *et al.*, 1981). *Arthrobacter* and *Pseudomonas* contain roughly 10% of their S in an oxidized form regardless of whether they are grown at low or high levels of S substrate (Table 15.7). Fungi such as *Fusarium* and *Trichoderma* show a similar percentage when grown at low substrate S levels;

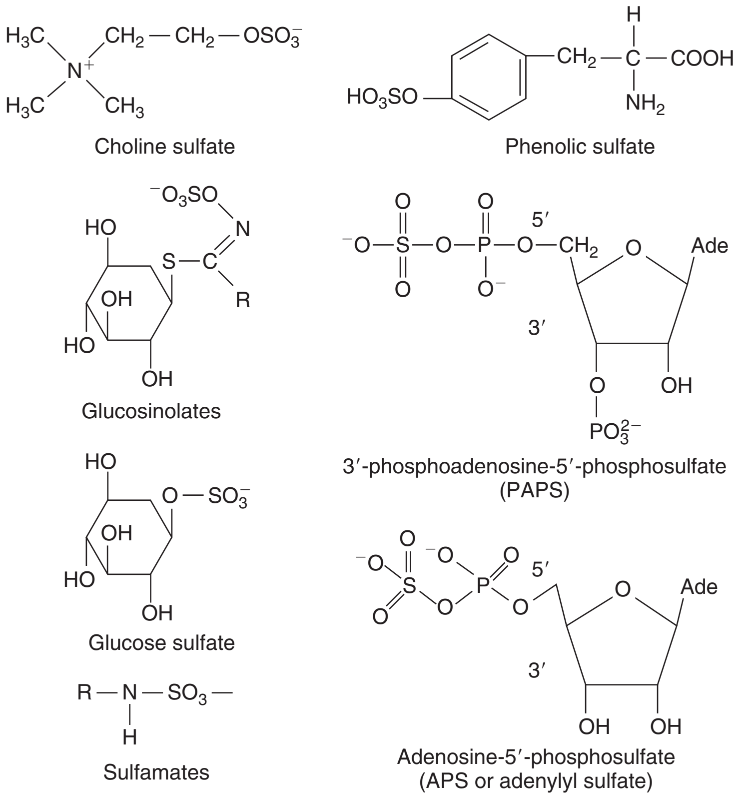



FIGURE 15.7 Ester sulfate forms of organic sulfur found in soil and as constituents of organisms.

TABLE 15.7 Total and HI-Reducible (Ester) Sulfur of Selected Microorganisms Grown in Cultures with Varying Sulfur Concentrations[a]

| | Total S ($\mu$g g$^{-1}$) | | | HI-reducible (ester) S (%) | | |
|---|---|---|---|---|---|---|
| Organism | 1[b] | 4 | 16 | 1[a] | 4 | 16 |
| *Arthrobacter globiformis* | 928 | 1123 | 1355 | 10 | 11 | 7 |
| *Pseudomonas cepacia* | 1108 | 1341 | 1339 | 13 | 10 | 12 |
| *Fusarium solani* | 1013 | 2318 | 2772 | 12 | 24 | 42 |
| *Trichoderma harzianum* | 852 | 1262 | 2034 | 6 | 17 | 37 |

[a] With permission from Saggar *et al.* (1981).

[b] The numbers 1, 4, and 16 represent the concentration of sulfur added to the growth medium in $\mu$g S ml$^{-1}$ of culture.

however, roughly 40% is in the ester form when they are grown at high levels of S availability. Fungi have been reported to store intracellular S as choline sulfate, but otherwise little is known about the microbial storage of ester S compounds. Fungi retain most of the ester S they synthesize, while bacteria release a large proportion

of the ester S into the culture medium. Sulfolipids, which represent another form of ester sulfate, have been reported for a limited number of bacteria. Algal sulfate esters also exist; the well-known algal compound, agar, is a sulfuric acid ester of a linear galactan. Other forms of organic S, such as phenyl sulfates and elemental S, have been found in a variety of soil organisms. Elemental S has been identified in the sporocarps of ectomycorrhizal fungi as well as in other self-inhibited and dormant structures. It is deposited by some of the bacteria capable of utilizing $H_2S$ as an electron donor during photosynthesis.

The C:S ratio in SOM is not as consistent as is the C:N ratio. Major differences are found due to type of parent material, leaching, and S inputs. A worldwide C:N:S ratio could be considered as 130:10:1. Agricultural and grassland soils average 90:8:1; luvisols and spodosols under forest conditions can range up to 200:12:1. Wide ratios are found in areas of low S supply, and fertilization with S or atmospheric deposition raises the soil S level.

## BIOLOGICAL IMPORTANCE OF SULFUR

Sulfur is an element essential to life. Sulfur-containing amino acids maintain the secondary, tertiary, and quaternary structures of proteins via disulfide linkages. Many enzymes are inhibited when treated with reagents that destroy sulfhydryl groups. The sulfhydryl groups are also involved in binding of substrates to enzymes. Reduced forms of S can serve as energy sources or source of reducing power for some prokaryotes. Oxidized forms, especially sulfate, can serve as terminal electron acceptors during anaerobic respiration.

## MICROBIAL TRANSFORMATIONS OF SULFUR

### Mineralization

Carbon-bonded S is mineralized through various pathways: (1) direct aerobic mineralization during the oxidation of C as an energy source, (2) anaerobic mineralization of organic matter (desulfurization), (3) incomplete oxidation of organic S into inorganic S compounds, (4) biological oxidation of $H_2S$ to sulfate via elemental S and sulfite, (5) biological oxidation of tetrathionate to sulfate via sulfide, (6) hydrolysis of cysteine by cysteine desulfhydrolase, and (7) indirect (enzymatic) mineralization when sulfate esters are hydrolyzed by sulfatases (Lawrence, 1987). The hydrolysis of ester sulfates occurs by splitting the O–S bond, through the action of sulfatase enzymes:

$$\text{R-O-SO}_3^- + H_2O \rightarrow ROH + H^+ + SO_4^{2-}.$$

There are numerous sulfatases, characterized by high specificity. They include arylsulfatases and choline sulfatases, whose production is repressed in the presence of available $SO_4^{2-}$. The sulfatases are bound to the cell walls of fungi and gram-positive bacteria, while in gram-negative bacteria, they are found in the periplasm.

### Immobilization

Inorganic S is usually assimilated into organic compounds as $SO_4^{2-}$ by plants and most microorganisms. This involves a series of enzymatic reactions called assimilatory $SO_4^{2-}$ reduction. In the first step, a permease enzyme participates in the transfer of $SO_4^{2-}$ across the cell membrane. This step requires the input of energy via ATP to form adenosine 5′-phosphosulfate (APS) and is catalyzed by ATP sulfurylase:

$$\text{ATP} + SO_4^{2-} \xrightarrow{\text{ATP sulfurylase}} \text{APS} + \text{PP}_i.$$

Another ATP is then used to form PAPS using APS kinase:

$$\text{APS} + \text{ATP} \xrightarrow{\text{APS kinase}} \text{PAPS} + \text{ADP}.$$

From here, two pathways can be used to form cysteine. In one, reductive enzymes form the unstable intermediate $SO_3^{2-}$. This is reduced by NADH to $HS^-$, which reacts with serine to produce cysteine:

$$\text{PAPS} + 2e^- \xrightarrow{\text{PAPS reductase, NADH}} SO_3^{2-} + \text{PAP}$$
$$SO_3^{2-} + 7H^+ + 6e^- \xrightarrow{SO_3^{2-}\text{ reductase, NADH}} HS^- + 3H_2O$$
$$HS^- + \text{serine} \xrightarrow{\text{cysteine synthase}} \text{cysteine} + H_2O.$$

This sequence has been observed in bacteria such as *B. subtilis, Staphylococcus aureus*, and *Enterobacter aerogenes* and the fungus *A. niger.* In the second pathway, glutathione is used to transfer a S group from APS to *O*-acetylserine to form cysteine:

$$\text{serine} + \text{acetyl-SCoA} \rightarrow O\text{-acetylserine} + \text{CoASH},$$

$$O\text{-acetylserine} + H_2S \rightarrow \text{cysteine} + \text{acetate} + H^+.$$

This latter sequence has been observed in *Escherichia coli* and *Salmonella typhimurium*. The presence of available cysteine regulates the $SO_4^{2-}$ permease and the enzymes involved early in this pathway. This ensures that these energy-consuming reactions occur only in the absence of available S. The other S-containing amino acid (methionine) is produced via the aspartate family of amino acids and *trans*-sulfuration, leading to homocysteine, which is then converted to methionine. In bacteria, methionine is synthesized from cysteine.

Microbial decomposition of plant residues with C:S ratios >400:1 (≈0.1% S) results in immobilization of S. At C:S ratios <200:1 (0.2% S), S is released into the environment. Sulfur mineralization is less highly correlated with the degradation of C than is N mineralization. The proportion of HI-reducible and C-bonded S is not a good indicator of potentially mineralizable S, nor is the activity of the enzyme arylsulfatase.

**Oxidation**

In the presence of available electron acceptors, reduced forms of S are oxidized by both chemical and microbial pathways:

$$HS^- \rightarrow S^0 \rightarrow S_2O_3^{2-} \rightarrow S_4O_6^{2-} \rightarrow SO_4^{2-}.$$

A wide variety of organisms are capable of oxidizing S in a wide variety of environments (Table 15.8). As with all soil organisms, the classification of these is strongly affected by developing molecular techniques. The names of some of these organisms may change and new ones may be added, but the basic physiology and enzymes utilized will still apply. These organisms can be divided into three groups: (1) photoautotrophs, including species of green and purple sulfur bacteria; (2) chemolithotrophs, such as members of the thiobacilli; and (3) heterotrophs, including a wide range of bacteria and fungi. While the first two occur generally in extreme environments such as hot sulfur springs, the last groups are largely responsible for oxidizing $S^0$ in aerobic soils (Germida and Janzen, 1993).

Hydrogen sulfide oxidation has been observed in many unicellular and filamentous cyanobacteria (Garlick *et al.*, 1977). The filamentous cyanobacterium *Oscillatoria limnetica* can perform anaerobic photosynthesis and uses $HS^-$ as the electron acceptor. The ecological significance of phototrophic $HS^-$ oxidation is evident in the flexibility of cyanobacteria to adjust to changing environmental conditions of aerobiosis and anaerobic phototrophy in HS-rich environments. Depending on the environmental conditions present, the resulting reductant is transferred to: (1) $CO_2$ to yield cell material, (2) protons to yield $H_2$ in the absence of $CO_2$, or (3) $N_2$ to yield ammonia when combined N is absent. The green sulfur and purple sulfur bacteria represent a diverse morphological group, including cocci, vibrio, rods, spirals, and budding and gliding organisms. Both groups are commonly found in mud and stagnant waters containing $H_2S$ and exposed to light. They are also found under extreme conditions of high salinity or temperature and occur in S hot springs and saline lakes as a colored layer under salt deposits. The green S bacteria are strictly anaerobic and obligately phototrophic. They are able to use $HS^-$ and $S^0$ as photosynthetic electron donors, which are oxidized to $SO_4^{2-}$. Thiosulfate ($S_2O_3^{2-}$) is also oxidized by the green S bacteria *Chlorobaculum thiosulfatophilum* (formerly *Chlorobium limicola* subsp. *thiosulfatophilum*). These organisms have external S granules in the presence of $HS^-$ and a homogeneous 16S rRNA sequence only distantly related to the purple S bacteria. The purple S bacteria are Proteobacteria ranging in color from bluish violet through purple, deep red, and orange because of the various carotenoid pigments that dominate the bacteriochlorophylls in color. These bacteria have a distinctive 16S rRNA composition that is shared by the nonphototrophic S oxidizers such as *Beggiatoa* and *Thiobacillus.* The phototrophic Proteobacteria include the Chromatiaceae, Ectothiorhodospiraceae, and Rhodospirillaceae families. The Chromatiaceae genera *Thiospirillum* and *Thiocapsa* store elemental S internally in the presence of $HS^-$. *Allochromatium vinosum* (formerly *Chromatium vinosum*) is a facultative

**TABLE 15.8** Characteristics of Some Sulfur-Oxidizing Archaea and Bacteria[a]

| Organism | Metabolism[b] | Inorganic substrates[c] | pH | Temp. (ºC) | Enzymes involved |
|---|---|---|---|---|---|
| *Acidianus ambivalens* | OL | $S^{2-}$, $S^0$, $H_2$ | 2.5 | 94 | Sulfur oxygenase/reductase |
| *Acidianus brierleyi* | OL | $S^{2-}$, $S^0$, TS, TT, $H_2$ | 1.8 | 70 | Sulfur oxygenase |
| *Oscillatoria limnetica* | OP | $S^{2-}$ | 7.5 | 35 | Hydrogen sulfide:quinone reductase |
| *Chlorobaculum thiosulfatophilum* | OP | $S^{2-}$, $S^0$, TT, $H_2$ | 6.8 | 25–35 | Flavocytochrome *c*, thiosulfate: cytochrome *c* reductase |
| *Allochromatium vinosium* | FP | $S^{2-}$, $S^0$, $SO_3^{2-}$, TS, $H_2$ | 7.0 | 30–35 | APS reductase, flavocytochrome *c*, thiosulfate:cytochrome *c* reductase |
| *Thiocapsa roseopersicina* | FP | $S^{2-}$, $S^0$, TS, $H_2$ | 7.3 | 20–35 | Sulfite:acceptor oxidoreductase |
| *Paracoccus denitrificans* | FL | $S^{2-}$, $S^0$, TS, $H_2$ | 8.0 | 30 | Thiosulfate-oxidizing enzyme system, sulfite dehydrogenase |
| *Paracoccus versutus* | FL | $S^{2-}$, $S^0$, TS | 8.0 | 30 | Thiosulfate-oxidizing enzyme system, sulfite dehydrogenase |
| *Acidiphilium acidophilum* (formerly *Thiobacillus acidophilus*) | FL | $S^{2-}$, $S^0$, $SO_3^{2-}$, TS, TT | 2.5 | 30 | Tetra-, trithionate hydrolase, thiosulfate dehydrogenase |
| *Thiobacillus denitrificans* | OL | $S^{2-}$, $S^0$, $SO_3^{2-}$, TS, TT | 7.0 | 30 | APS reductase, sulfite oxidase |
| *Halothiobacillus neapolitanus* | OL | $S^{2-}$, $S^0$, $SO_3^{2-}$, TS, TT | 7.0 | 30 | |
| *Starkeya novellus* (formerly *Thiobacillus novellus*) | FL | $S^{2-}$, $S^0$, $SO_3^{2-}$, TS, TT | 7.0 | 30 | Sulfite:cytochrome *c* oxidoreductase |
| *Acidithiobacillus ferrooxidans* | OL | $S^{2-}$, $S^0$, $SO_3^{2-}$, TS, TT, $H_2$ | 2.5 | 30 | Sulfur:Fe(III) oxidoreductase, sulfide:Fe(III) oxidoreductase |
| *Thermithiobacillus tepidarius* | OL | $S^{2-}$, $S^0$, $SO_3^{2-}$, TS, TT | 7.0 | 44 | Trithionate hydrolase, tetrathionate synthase, sulfite dehydrogenase |
| *Acidithiobacillus thiooxidans* | OL | $S^{2-}$, $S^0$, $SO_3^{2-}$, TS, TT | 2.5 | 30 | Sulfite:cytochrome *c* oxidoreductase |

[a]With permission from Friedrich (1998).
[b]FL, facultative lithotroph; FP, facultative phototroph; OL, obligate lithotroph; OP, obligate phototroph.
[c]TS, thiosulfate; TT, tetrathionate.

photoautotroph, able to grow anaerobically in the light with $HS^-$, $SO_3^{2-}$, $S_2O_3^{2-}$, and $H_2$, but not $S_4O_6^{2-}$. It is also capable of aerobic chemotrophic growth in the dark with $S_2O_3^{2-}$ and $CO_2$, provided the oxygen concentration is low. Sulfur oxidation by this organism proceeds in three steps: (1) oxidation of $HS^-$ or $S_2O_3^{2-}$ to $S^0$, which is deposited intracellularly; (2) oxidation of $HS^-$ or $S^0$ to $SO_3^{2-}$; and (3) oxidation of $SO_3^{2-}$ to the final product, $SO_4^{2-}$. Members of the family Ectothiorhodospiraceae transiently store elemental S outside the cell and subsequently oxidize it to $SO_4^{2-}$ upon depletion of $HS^-$. Some of the species in this family are marine, slightly halophilic curved gram-negative motile cells. Other species grow only in extremely salty alkaline conditions.

A wide, unrelated array of diverse chemolithitrophs have been grouped on the basis of their sulfur oxidation, including bacteria such as *Thiobacillus, Beggiatoa*, *Thioploca,* and *Thiothrix* and the archaeon *Acidianus. Acidianus ambivalens* (formerly *Desulfurolobus ambivalens*) exhibits the unique combination of dissimilatory aerobic S oxidation and anaerobic S respiration in one strain. The organism is a strictly acidophilic, extreme thermophilic, and obligately chemolithotrophic archaeon able to obtain energy from aerobic S oxidation and also grows under anaerobic conditions by reducing $SO_4^{2-}$ with $H_2$ as the electron donor. The thiobacilli represent the classic S-oxidizing organisms. The historical criterion for classifying organisms into the *Thiobacillus* genus was that all the species are rod-shaped eubacteria able to obtain energy for autotrophic growth by oxidizing inorganic S. Widespread application of 16S rRNA gene sequence analysis and DNA–DNA hybridization have provided tools for clarifying the taxonomy of this genus. Application of these tools has resulted in the reassignment of 14 species of thiobacilli into other genera (Kelly and Wood, 2000).

The majority of the thiobacilli are obligate aerobes, though some such as *T. denitrificans* can grow anaerobically by using nitrate as a terminal electron acceptor. Others use electron donors such as $Fe^{2+}$ (*Acidithiobacillus ferrooxidans*) or $NCS^-$ (*Thiobacillus thioparus*), rather than S. The fact that they are facultative or obligate chemolithotrophs means they are able to oxidize S independent of the supply of available C. These bacteria are easily isolated from extreme environments such as hot, acid soils, S-polluted soils near sulfur piles, and soils subjected to high atmospheric deposition of S. Attempts to isolate these bacteria from agricultural soils have been more sporadic. The view that thiobacilli are the dominant players in S oxidation in soils is largely based on the observation that these bacteria are capable of much higher rates of S oxidation in culture than those achieved by heterotrophs growing under similar conditions. However, the isolation of these organisms from agricultural soils has not been very successful, and no consistent correlation between S oxidation rates and the presence of thiobacilli has been found except that these rates are generally low in soils that lack these organisms.

Sulfur oxidation is also mediated by a large number of heterotrophic soil microorganisms including bacteria such as *Arthrobacter*, *Bacillus*, *Micrococcus*, *Mycobacterium*, and *Pseudomonas*; some actinomycetes; and fungi such as *Absidia*, *Alternaria*, *Fusarium*, and *Trichoderma.* Lawrence and Germida (1991)

divided these organisms into those that oxidized $S^0$ to produce primarily thiosulfate, those that oxidized $S^0$ to produce sulfate, and those capable of oxidizing thiosulfate to sulfate. The first group was found to be the most abundant population. No energy is gained by these organisms and the transformations appear to be incidental to the major metabolic pathways. The reason for the oxidation is, therefore, not known; but the possibility of protection against $H_2O_2$ has been suggested. It remains difficult to partition S oxidation between autotrophic and heterotrophic populations, and it is likely a mixed population that is responsible in most instances.

**Reduction**

Reduction of oxidized forms of S, particularly $SO_4^{2-}$, by microorganisms occurs in two different ways. In the first, S is incorporated into cellular constituents such as the S in amino acids. This process is referred to as assimilatory sulfate reduction, or immobilization, as described above. In the other, the reduction leads to the formation of sulfide (e.g., $H_2S$) as the end product. This is referred to as dissimilatory, or respiratory, sulfate reduction. This process is mediated by anaerobic, organotrophic organisms that use low-molecular-weight organic compounds or $H_2$ as electron donors and the oxidized S compounds as terminal electron acceptors in a process similar to denitrification. These organisms are responsible for sulfide formation in waterlogged soils and sediments. Historically, the organisms involved in dissimilatory S or $SO_4^{2-}$ reduction were thought to represent a narrow physiological and ecological group that belonged to either *Desulfovibrio* or *Desulfotomaculum.* Sulfate reduction is now recognized in a number of bacterial genera (Castro *et al.*, 2000; Table 15.9). Sulfate-reducing bacteria are found over an extensive range of pH and salt concentrations in saline lakes, evaporation beds, deep-sea sediments, and oil wells. The organisms can tolerate heavy metal and dissolved sulfide concentrations up to 2%. Because it is mediated mainly by anaerobic bacteria, sulfate reduction is not important in well-aerated soils, except in anaerobic microsites, but is a major component of the S cycle in periodically waterlogged or flooded soils such as rice paddies (Germida *et al.*, 1992).

Two groups of sulfate-reducing bacteria have been recognized (Germida *et al.*, 1992). The first group consists of bacteria that use organic C as an energy source, but do not completely oxidize it to $CO_2$. This group includes species of *Desulfovibrio* and *Desulfotomaculum*, whose principle metabolic products are acetate and $H_2S$, but some strains can also grow fermentatively on pyruvate in the absence of sulfate. The second group is more diverse and includes species of *Desulfobacter*, *Desulfococcus*, *Desulfosarcina*, and *Desulfonema.* These species can completely oxidize organic C to $CO_2$ by using $SO_4^{2-}$ as the terminal electron acceptor.

Although sulfate-reducing bacteria are largely organotrophic, such that most of the C fixed is derived from organic matter, some organic molecules such as low-molecular-weight fatty acids (e.g., butyric, propionic, and acetic acids) are inhibitory

**TABLE 15.9** Characteristics of Some Genera of Dissimilatory Sulfate-Reducing Bacteria[a]

| Genus | Morphology | Growth temp. (°C) | Substrate/comments |
|---|---|---|---|
| Gram-negative mesophilic | | | |
| *Desulfobulbus* | Lemon to rod | 25–40 | Incomplete oxidation of acetate |
| *Desulfomicrobium* | Ovoid to rod | 25–40 | Incomplete oxidation of acetate |
| *Desulfomonas* | Rod | 30–40 | Complete oxidation of benzoate and fatty acids |
| *Desulfovibrio* | Spiral to vibroid | 25–40 | Incomplete oxidation of lactate; fatty acids may be completely oxidized |
| *Desulfobacter* | Oval to rod | 20–33 | Incomplete oxidation of lactate and propionate |
| *Desulfococcus* | Spherical or lemon | 28–35 | Acetate completely oxidized |
| *Desulfomonile* | Rod | 37 | Acetate completely oxidized |
| *Desulfonema* | Filaments | 28–32 | Acetate completely oxidized |
| *Desulfosarcina* | Oval rods or coccoid, packages | 33 | Complete oxidation of fatty acids |
| Gram-positive spore-forming | | | |
| *Desulfotomaculum* | Straight to curved rods | Most 25–40<br>Some 40–65 | Lack desulfoviridin, the usual replacement is P-582 |
| Bacterial thermophilic | | | |
| *Thermodesulfobacterium* | Vibroid to rod | 65–70 | Incomplete oxidation of acetate |
| Archaeal thermophilic | | | |
| *Archaeoglobus* | Coccoid | 64–92 | Incomplete oxidation of acetate |

[a] With permission from Castro *et al.* (2000).

to growth. In such cases, only certain organic substrates such as lactic and pyruvic acids are utilized as electron donors. These reactions are as follows:

$$\text{lactic acid, } 2\ CH_3CHOHCOOH + SO_4^{2-} \rightarrow 2\ CH_3COOH + S^{2-} + 2\ CO_2 + H_2O;$$

$$\text{pyruvic acid, } 4\ CH_3COCOOH + SO_4^{2-} \rightarrow 3\ CH_3COOH + CH_3COO^- + HS^- + 4\ CO_2.$$

The pathway for dissimilatory reduction uses the same activation of $SO_4^{2-}$ to APS as found in assimilatory reduction (see Microbial Transformations of Sulfur—Immobilization). The dissimilatory reduction scheme includes the production of $HSO_3^-$ without the involvement of PAPS, and the other difference is the large production of $HS^-$ without the formation of organic S.

Aside from their obvious importance in the S cycle, sulfate-reducing bacteria are important regulators of a variety of processes in anaerobic upland and wetland

soils, including organic matter turnover, biodegradation of chlorinated aromatic pollutants, and mercury methylation.

### Volatilization

The decomposition of organic S compounds in poorly drained soils, sediments, manures, and organic wastes can lead to the formation of volatile organic S compounds. These compounds include mercaptans such as methyl mercaptan ($CH_3SH$) and alkyl sulfides such as dimethyl sulfide ($CH_3SCH_3$). Volatile S compounds may be of importance because they can inhibit plant growth, nitrification, and other biochemical processes. These compounds are also undesirable atmospheric pollutants causing unpleasant odors and adversely affecting climate.

## MICRONUTRIENTS AND TRACE METALS

Micronutrients are chemical elements that are required by plants and microorganisms in small amounts. These micronutrients are vital constituents of enzymes or growth hormones. The essential trace elements for plants are iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), boron (B), molybdenum (Mo), and nickel (Ni). Microorganisms and higher animals also require copper (Cu), cobalt (Co), chromium (Cr), selenium (Se), and tin (Sn). Other metal elements such as mercury (Hg), arsenic (As), lead (Pb), and cadmium (Cd) are not essential to organisms, but are found in soils as potentially toxic contaminants. This section will focus on select micronutrients and nonessential metal elements, which will be referred to collectively as trace metals for simplicity, even though some of these elements (particularly Fe) can exist in soils at much higher than trace concentrations.

## MICRONUTRIENT AND TRACE METAL CYCLING IN SOIL

A generic cycle diagram for micronutrient and trace metals in soils is illustrated in Fig. 15.8. Concentrations of elements as free ions or soluble complexes are strongly influenced by abiotic reactions such as changes in oxidation state, fixation to mineral surfaces, complexation with organic matter, and formation of insoluble minerals. Microorganisms are able to solubilize minerals and change the soil redox potential and pH. Plant availability of micronutrients is therefore highly dependent on microbial activity. Recycling of micronutrients occurs when plant litter is returned to the soil and mineralized by the microbial biomass, thereby releasing the trace metals. The production and secretion of various chelating agents by plant roots and microorganisms promote the dissolution and weathering of minerals and facilitate the movement of micronutrients to roots. Inputs of most heavy metal elements are from anthropogenic sources. Human activities since the late 1800s have released at least $10^6$ metric tons of Hg into the environment. Coal contains 1200 to 21,000 ng Hg $g^{-1}$, and the burning of oil and coal releases 2 to

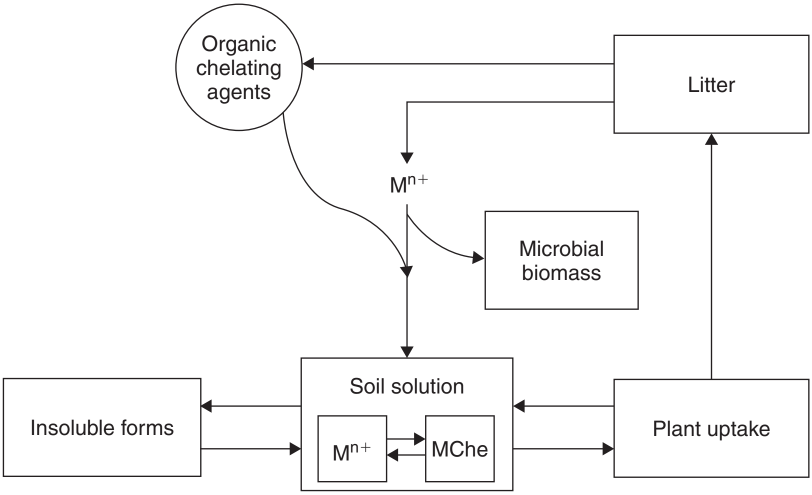



FIGURE 15.8 A generic cycle diagram for micronutrient and metal elements in soil (with permission from Stevenson and Cole, 1999).

$7 \times 10^4$ metric tons Hg year$^{-1}$. Most of this Hg eventually finds its way into the oceans. An additional transformation that many potentially toxic metals undergo, resulting in their loss from the soil system, and that some micronutrient metals do not, is volatilization. This transformation is a detoxification strategy for organisms and releases the toxic metal to the atmosphere in a volatile form.

## NATURE AND FORMS IN SOIL

Trace metals in soil originate from (1) parent materials from which the soils are formed, (2) contamination from impurities in soil amendments such as mineral fertilizers or biosolids, and (3) atmospheric deposition of natural (e.g., wind-eroded soil or volcanic materials) or anthropogenic (e.g., fossil fuel combustion products) particulates. The main source of trace metals is the parent material because the amounts added, even in high fertilizer use agroecosystems, are generally too low to have an influence on the total amount present. Some sites that have received high rates of biosolid amendments over a long period of time may have soil concentrations of trace metals many times higher than that of the geochemical background (Table 15.10).

Micronutrients and trace metals in soil can be water-soluble ions or water-soluble complexes with organic or inorganic ligands. They may be present as exchangeable cations on clay mineral surfaces. Some trace elements (e.g., $Cu^{2+}$) are retained by clay minerals or oxides even in the presence of an excess of $Ca^{2+}$. Trace elements bound in this manner are called "specifically adsorbed." Organic matter is also a substrate from adsorption and complexation of trace metals. And finally, trace

TABLE 15.10 Mean Concentrations of Some Trace Metals in Igneous and Sedimentary Rocks[a]

| | Mean concentration (μg g$^{-1}$) | | | | |
|---|---|---|---|---|---|
| | Igneous rocks | | Sedimentary rocks | | |
| Element | Granite | Basalt | Shale | Sandstone | Carbonate |
| Fe | 13,700 | 77,600 | 47,000 | 9800 | 3800 |
| Zn | 45 | 86 | 95 | 16 | 20 |
| Mn | 195 | 1280 | 850 | — | 1100 |
| Cu | 13 | 110 | 45 | — | 4 |
| Pb | 48 | 7.8 | 20 | 7 | 9 |
| Mo | 6.5 | 0.51 | 2.6 | 0.2 | 0.4 |
| Hg | 0.1 | 0.2 | 0.4 | 0.3 | 0.2 |

[a] With permission from Mason and Moore (1982).

metals may exist in sparingly soluble or insoluble mineral forms, including occlusion in Fe or Mn oxides or as cations that have undergone isomorphous substitution in silicate clays. Generally, soluble and exchangeable pools of micronutrients represent a small proportion of the total, while the large majority remains in specifically adsorbed or bound pools. These pools are usually in equilibrium with each other such that if the micronutrient concentration is soil solution decreases due top plant uptake or leaching, insoluble forms may release the micronutrient.

Complexation of soluble metal ions with insoluble organic matter reduces bioavailability, whereas the formation of soluble organic complexes increases bioavailability. Plant roots and microorganisms in the associated rhizosphere produce and secrete large quantities of organic acids and other biochemical chelating agents (Stevenson, 1991). These chelating agents are able to form stable complexes with metal ions. These chelating compounds range from simple amino acids to humic and fulvic complexes and represent a significant proportion of the metal binding capacity of soils.

## BIOLOGICAL IMPORTANCE

Iron is required for nutrition by all organisms, prokaryotic and eukaryotic alike, with a small group of homolactic fermenting bacteria being the only known exception. It is needed in enzymatic processes during aerobic or anaerobic respiration that involve the transfer of electrons to a terminal acceptor. Photosynthesizers require Fe for ferrodoxin, which is part of the photosynthetic pathway. Ferrous iron ($Fe^{2+}$) can be used as a major energy source by some bacteria, while ferric iron ($Fe^{3+}$) can be used as a terminal electron acceptor under different conditions by the same or other bacteria. $Fe^{2+}$ may have served as an important reductant during the evolution of photosynthesis as a scavenger of the toxic oxygen produced

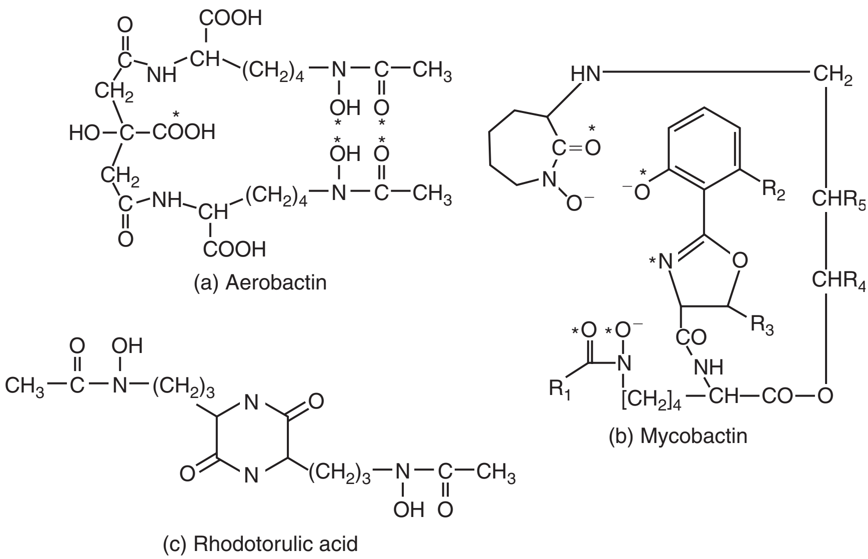



FIGURE 15.9 Chemical structures of two bacterial siderophores, (a) aerobactin and (b) mycobactin, and one fungal siderophore, (c) rhodotorulic acid. Iron chelation sites are indicated by asterisks (from Neilands, 1981).

during the process before organisms developed the ability to produce superoxide dismutases for this purpose. Since microorganisms cannot take up insoluble forms of Fe into their cells, and since the solubility of $Fe^{3+}$ in soil solution is low, soil bacteria and fungi have developed the ability to produce $Fe^{3+}$-chelating compounds called siderophores (Fig. 15.9). Siderophores help keep $Fe^{3+}$ in solution and permit the uptake into microbial cells. After transport into the cell, the chelated $Fe^{3+}$ is usually reduced enzymatically to $Fe^{2+}$ and released from the siderophore, which itself can be released by the cell for further scavenging of iron.

Several other micronutrient metal elements are required by microorganisms, plants, and animals as part of the structure of a number of enzymes (Table 15.11). Manganese is required in photosynthesis, where it plays a role in the production of oxygen in photosystem II. It can also serve as an energy source for some bacteria and also as a terminal electron acceptor. Molybdenum is an essential part of a number of enzyme structures such as nitrogenase, nitrate reductase, and sulfite reductase and may also act as an effective inhibitor of sulfate reduction. Selenium is an essential component together with Mo in the structure of formate dehydrogenase in several bacterial species. Nonessential metal elements such as Hg, As, Pb, and Cd may not be required nutritionally, but many microorganisms have acquired the ability to alter these metals enzymatically in their environments as a means to combat potentially toxic concentrations. These elements also bioaccumulate in the tissues of higher animals and therefore pose potential human health risks.

TABLE 15.11 Some Micronutrients and Their Functions in Microorganisms[a]

| Element | Soluble ionic form | Function in microbial metabolism |
|---|---|---|
| Fe | $Fe^{2+}$, $Fe^{3+}$ | Present in cytochromes, ferredoxins, and other iron–sulfur proteins; cofactor of enzymes |
| Zn | $Zn^{2+}$ | Present in alcohol dehydrogenase, alkaline phosphatase, adolase, and RNA and DNA polymerase |
| Mn | $Mn^{2+}$ | Present in bacterial and mitochondrial superoxide dismutase and in photosystem II; cofactor of some enzymes |
| Mo | $MoO_4^{2+}$ | Present in nitrate reductase, nitrogenase, xanthine dehydrogenase, and formate dehydrogenase |
| Se | $SeO_3^{2-}$ | Present in glycine reductase and formate dehydrogenase |
| Co | $Co^{2+}$ | Present in coenzyme $B_{12}$-containing enzymes |
| Cu | $Cu^{2+}$ | Present in cytochrome oxidase, nitrite reductase of denitrifying bacteria, and oxygenases |
| Ni | $Ni^{2+}$ | Present in urease, hydrogenase, and factor $F_{430}$ |

[a] With permission from Gottschalk (1986).

## MICROBIAL TRANSFORMATIONS

### Oxidation and Reduction

Biologically mediated reduction–oxidation reactions are often associated with energy production by the organism. Reduced inorganic compounds are oxidized by chemoautotrophs to generate electrons used in ATP production, while oxidized compounds are reduced during energy production under anaerobic conditions when the element in question acts as an alternative terminal electron acceptor.

*Oxidation of Fe and Mn.* Microorganisms are involved in the oxidation of Fe, but not all Fe oxidation is microbially mediated. Ferrous iron ($Fe^{2+}$) is rapidly oxidized chemically in aerated solution at pH >5. It is therefore difficult to demonstrate conclusively the microbial role of Fe oxidation under neutral or alkaline pH conditions. The best evidence for microbially mediated Fe oxidation is from acidophilic bacteria operating at pH <5. The most commonly studied acidophilic iron-oxidizing organism is *Acidithiobacillus ferrooxidans* (formerly *Thiobacillus ferrooxidans*). It is easily cultivated and has been the focus of extensive genetic and physiological studies (Rawlings, 2001). It is a gram-negative, motile rod that derives energy and reducing power from the oxidation of $Fe^{2+}$, reduced forms of S, metal sulfides, $H_2$, and formate. It uses $CO_2$ as its C source and preferentially $NH_3$–N as its N source, though it can also use $NO_3$–N and some strains can fix $N_2$. *Ac. ferrooxidans* is mesophilic, growing in the range of 15 to 42ºC with an optimum in the range 30–35ºC. Other acidophilic iron oxidizers include *Leptospirillum ferroooxidans, Sulfolobus* spp., and *Acidianus* spp. *Leptos. ferrooxidans* oxidizes iron for energy but cannot oxidize reduced S. The best known examples of extreme thermophilic iron-oxidizing bacteria are *Sulfolobus acidocaldarius* and *Acidianus brierleyi* (formerly *Sulfolobus brierleyi*), both of which belong to the

Archaea. Their temperature range is between 55 and 90°C, with an optimum in the range 70–75°C. Sulfur is their usual electron donor, but it can be replaced by $Fe^{2+}$.

*Gallionella ferruginea* represents the strongest evidence for enzymatic oxidation of Fe under neutral pH conditions; however, it is equivocal. *Gallionella* are stalked bacteria that use $Fe^{2+}$ as their electron donor and $CO_2$ as their C source. The bean-shaped cells secrete colloidal $Fe(OH)_3$, without an organic matrix. They are aerobic and occur in Fe-containing fresh or marine waters. Other bacteria that have been associated with Fe oxidation include sheathed bacteria such as *Sphaerotilus*, *Leptothrix*, and "*Clonothrix*." However, these bacteria are more likely iron-depositing bacteria rather than Fe oxidizers in that they bind already oxidized iron at their cell surfaces. The distinction between abiotic and microbially mediated Fe oxidation is difficult as the physicochemical properties of biogenic iron oxides are similar to those of their abiotic counterparts (Fortin and Langley, 2005), and therefore it remains difficult to determine the exact role of microorganisms in their formation.

Bacterial oxidation of $Mn^{2+}$ occurs in both soils and sediments. On ocean bottoms, microorganisms are responsible for the formation of ferromanganese nodules. The chemical oxidation of $Mn^{2+}$ occurs only above pH 8, and therefore Mn oxidation in neutral and acidic environments is microbially mediated. A large number of bacterial and fungal groups are participants. Three phylogenetically distinct organisms have been studied extensively for Mn oxidation: *Leptothrix discophora*, *Pseudomonas putida* strains MnB1 and GB-1 (later synonym for *Arthrobacter siderocapsulatus*), and a *Bacillus* sp. strain (Tebo *et al.*, 2004). All three organisms oxidize Mn enzymatically on an exopolymer matrix surrounding the cell: *Leptot. discophora* on an extracellular sheath, *Ps. putida* on the outer membrane glycocalyx, and *Bacillus* on the exosporium (Fig. 15.10). Molecular genetic

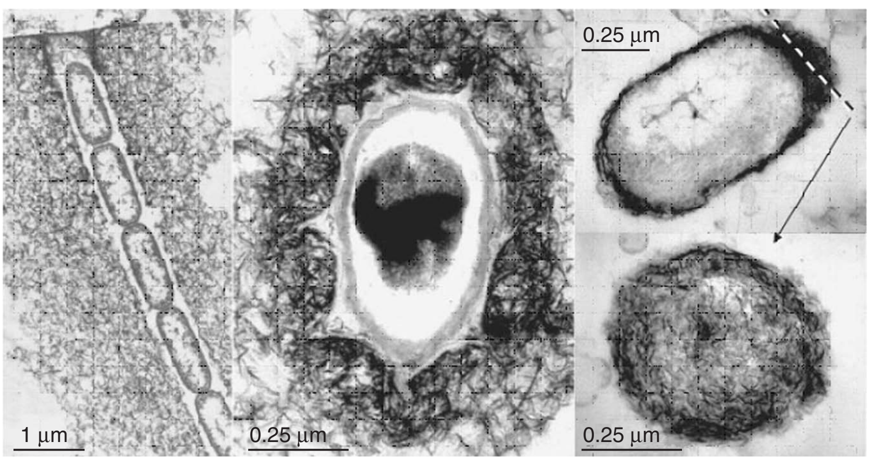



FIGURE 15.10 Transmission electron micrographs of representatives of the three Mn-oxidizing bacteria (with permission from Tebo *et al.*, 2004). Left, an unidentified *Leptothrix* sp.; center, spores of the marine *Bacillus* sp. strain SG-1; right, *Pseudomonas putida* strain MnB1.

techniques have revealed that all three organisms possess genes that are involved in Mn oxidation and that share sequence similarity with multicopper oxidase enzymes. Other Mn oxidizers, such as *Leptothrix pseudoochracea*, will oxidize dissolved $Mn^{2+}$ with catalase enzymes that utilize hydrogen peroxide ($H_2O_2$).

Some rhizosphere bacteria oxidize Mn and deposit $MnO_2$ on the outside of the root. Examination shows the root coated with a black precipitate. The easiest method of control is by genetic selection of the host plant, making it incompatible for the bacteria. Manganese toxicity from excess available Mn can occur in acidic soils. The precipitation of Mn in the filaments of a mycorrhizal fungus has been found to allow the growth of Mn-sensitive plants in such soils.

*Reduction of Fe and Mn.* Enzymatic Fe or Mn reduction occurs as part of anaerobic respiration in which the oxidized form serves as the dominant or exclusive terminal electron acceptor. It may also accompany fermentation in which the metal serves as a supplementary electron acceptor. Both processes are referred to as dissimilatory iron reduction. When Fe or Mn is reduced during cellular uptake for incorporation into cellular components, the process is referred to as assimilatory iron or manganese reduction. Dissimilatory reduction is the dominant process and will be the focus of this discussion.

Iron reduction in the form of anaerobic respiration is an important means of mineralization of organic matter in low-$O_2$ environments where sulfate and nitrate occur in amounts insufficient to support sulfate or nitrate respiration. A wide range of Archaea and bacteria are able to conserve energy through the reduction of ferric iron ($Fe^{3+}$) to ferrous iron ($Fe^{2+}$), and many of the same organisms are also able to grow through the reduction of $Mn^{4+}$ to $Mn^{2+}$ (Table 15.12). The environmental significance of these processes is that Fe-reducing communities can be responsible for most of the organic matter oxidation in many subsurface environments, and several xenobiotics can be degraded under anaerobic conditions by Fe and Mn reducers. Historically, studies on the reduction of $Fe^{3+}$ and $Mn^{4+}$ focused on organisms that grow predominantly by fermentation of sugars with metals used as minor electron acceptors, typically $<5\%$ of the reducing equivalents used for metal reduction. Metal reduction through this form of metabolism

TABLE 15.12A Microbially Mediated Oxidation Reactions of Metals in Soils and Sediments and Examples of Organisms Involved[a]

| Element | Half-reaction | Strategy[b] | Example organisms involved |
|---|---|---|---|
| Fe | $2Fe^{2+} \rightarrow 2Fe^{3+} + 2e^-$ | E | *Acidithiobacillus ferrooxidans* |
| Mn | $Mn^{2+} \rightarrow Mn^{4+} + 2e^-$ | E, D | *Arthrobacter*, *Pseudomonas* |
| Hg | $Hg^0 \rightarrow Hg^{2+} + 2e^-$ | NE | *Bacillus*, *Pseudomonas* |
| As | $AsO_2^- \rightarrow AsO_4^{3-} + 2e^-$ | D | *Bacillus*, *Pseudomonas*, *Alcaligenes faecalis* |
| Se | $Se^{2+} \rightarrow Se^0 + 2e^-$ | E | *Bacillus*, *Acidithiobacillus* |
| U | $U^{4+} + 2e^- \rightarrow U^{6+}$ | E | *Acidithiobacillus ferrooxidans* |

**TABLE 15.12B** Microbially Mediated Reduction Reactions of Metals in Soils and Sediments and Examples of Organisms Involved[a]

| Element | Half-reaction | Strategy[b] | Example organisms involved |
|---|---|---|---|
| Fe | $2Fe^{3+} + 2e^{-} \rightarrow 2Fe^{2+}$ | AR | *Geobacter, Desulfovibrio*, *Pseudomonas*, *Acidithiobacillus* |
| Mn | $Mn^{4+} + 2e^{-} \rightarrow Mn^{2+}$ | AR | *Geobacter*, *Shewanella*, *Desulfovibrio*, *Pseudomonas*, *Bacillus* |
| Hg | $Hg^{2+} + 2e^{-} \rightarrow Hg^{0}$ | D | *Pseudomonas*, *Streptomyces*; *Bacillus*, *Vibrio*, *Alcaligenes*, *Acinetobacter* based on Hg resistance |
| Se | $SeO_4^{2-} + 8e^{-} \rightarrow Se^{2-}$ | AR | *Pseudomonas*, *Flavobacterium*, *Citrobacter*, *Clostridium*, *Thiobacillus* |
| Cr | $Cr^{6+} + 3e^{-} \rightarrow Cr^{3+}$ | AR, D | *Pseudomonas*, *Aeromonas*, *Clostridium*, *Citrobacter*, *Bacillus*, *Streptomyces*, *Desulfovibrio* |
| U | $U^{6+} + 2e^{-} \rightarrow U^{4+}$ | AR | *Geobacter metallireducens*, *Shewanella putrefaciens*, *Desulfovibrio* |

[a] With permission from Silvia *et al.* (2005) with data from Ehrlich (1996), Frankenberger and Lossi (1995), and Lovley (1993).

[b] NE, not enzymatic; AR, element is terminal electron acceptor in anaerobic respiration; D, detoxification; E, energy source.

did not conclusively demonstrate increases in cell yield. *Shewanella oneidensis* (formerly *Alteromonas putrefaciens* and then *Shewanella putrefaciens*) and *Geobacter metallireducens* were then unequivocally shown to conserve energy for growth through the reduction of $Fe^{3+}$ or $Mn^{4+}$, and more recently, numerous organisms that can grow using $Fe^{3+}$ and $Mn^{4+}$ as electron acceptors have been isolated. Most organisms that are known to grow through the reduction of $Fe^{3+}$ or $Mn^{4+}$ are relatives of *Ge. metallireducens* and include the genera *Geobacter*, *Desulfuromonas*, *Desulfuromusa*, and *Pelobacter*. With the exception of the last group, these organisms are able to completely oxidize a wide range of organic compounds, including acetate, when respiring using $Fe^{3+}$ or $Mn^{4+}$. *Pelobacter* are restricted to ethanol, lactate, formate, and $H_2$.

The primary products of the metabolism of the fermentative $Fe^{3+}$-reducing microorganisms are typical fermentation acids, alcohols, and $H_2$. Most of the electron transfer to $Fe^{3+}$ during the metabolism of sugars and amino acids results from the oxidation of the fermentation products, and acetate is considered to be the most important fermentation product in $Fe^{3+}$-reducing environments. The $Fe^{3+}$ reducer *Ge. metallireducens* oxidizes acetate by the reaction

$$\text{acetate}^{-} + 8\,Fe^{3+} + 4\,H_2O \rightarrow 2\,HCO_3^{-} + 8\,Fe^{2+} + 9\,H^{+}.$$

*Ge. metallireducens* oxidizes various other volatile fatty acids and simple alcohols. *Sh. oneidensis* can also conserve energy to support growth by coupling the

oxidation of formate, which is oxidized to $CO_2$, or lactate or pyruvate, which are incompletely oxidized, to the reduction of $Fe^{3+}$:

$$\text{formate}^- + 2\,Fe^{3+} + H_2O \rightarrow HCO_3^- + 2\,Fe^{2+} + 2\,H^+,$$

$$\text{lactate}^- + 4\,Fe^{3+} + 2\,H_2O \rightarrow \text{acetate}^- + HCO_3^- + 4\,Fe^{2+} + 5\,H^+,$$

$$\text{pyruvate}^- + 2\,Fe^{3+} + 2\,H_2O \rightarrow \text{acetate}^- + HCO_3^- + 2\,Fe^{2+} + 3\,H^+.$$

*Ge. metallireducens* can also completely oxidize a wide variety of monoaromatic compounds, such as toluene, *p*-cresol, and phenol, to carbon dioxide with $Fe^{3+}$ serving as the sole electron acceptor.

Dissimilatory $Fe^{3+}$ reduction has a greater overall environmental impact than microbial reduction of any other metal. It has been implicated as an important process in the following phenomena: (1) organic matter decomposition in a variety of freshwater, estuarine, and marine sediments; (2) the decomposition of aromatic hydrocarbons in contaminated aquifers; (3) the control of the extent of methane formation in shallow freshwater environments; (4) the release of phosphate and trace metals into soil solution; (5) soil gleying; and (6) the corrosion of buried iron and steel pipes (see review by Lovley, 1991). Manganese reduction may also serve to (1) assist in the oxidation of organic matter in waters or sediments, (2) release dissolved Mn into groundwaters and sediments, and (3) release trace metals bound to Mn oxides.

*Reduction of other metals.* Several other metals are subject to microbially mediated reduction (Lloyd, 2003, Table 15.12). Several organisms have been isolated that can grow through dissimilatory reduction of $As^{5+}$ (e.g., *Sulfurospirillum arsenophilum*, a microaerobic S-reducing bacterium isolated from an As-contaminated watershed). The reduction of $AsO_4^{3-}$ to $AsO_2^-$ by the ArcC reductase enzyme also forms the basis for a microbial arsenic resistance mechanism. A wide range of facultative anaerobes are able to reduce $Cr^{6+}$ to $Cr^{3+}$, including *E. coli*, *Pseudomonas* spp., *Sh. oneidensis*, and *Aeromonas* spp. Obligate anaerobes are also able to reduce Cr enzymatically, and the reduction of Cr coupled with anaerobic growth has been observed in S-reducing bacteria.

Numerous microorganisms reduce $Hg^{2+}$ to $Hg^0$ for self-protection only, as little evidence exists that Hg reduction supports microbial growth. Reduction is linked to mercury-resistance (*mer*) operons providing a detoxification in which highly soluble $Hg^{2+}$ is reduced to volatile $Hg^0$. The reduction is for protection against the toxic metal rather than for the energy-conserving electron transport characteristics of other microbial reductions. The mercuric reductase is a flavin-containing disulfide that drives the following reaction:

$$Hg(RS)_2 + NADPH + H^+ \rightarrow Hg^0 + NADP^+ + 2RSH.$$

Organisms active in reducing Hg include strains of *Pseudomonas* spp., enteric bacteria, *S. aureus*, *Ac. ferrooxidans*, group B *Streptococcus*, *Streptomyces*, and *Cryptococcus.* Other organisms such as *Bacillus*, *Vibrio*, *Flavobacterium*,

TABLE 15.13 Microbial Genera Known to Contribute to Methylation of Metals under Aerobic Conditions[a]

| Genera | Arsenic (As) | Mercury (Hg) | Selenium (Se) | Lead (Pb) |
|---|---|---|---|---|
| Fungi | | | | |
| Aspergillus | X | X | X | |
| *Candida* | X | | | |
| *Neurospora* | X | X | | |
| *Penicillium* | X | | X | |
| *Saccharomyces* | X | X | | |
| *Scopulariopus* | X | X | X | |
| Bacteria | | | | |
| *Aeromonas* | X | | X | X |
| *Acinetobacter* | | | | X |
| *Bacillus* | | X | | |
| *Escherichia* | X | X | | |
| *Flavobacterium* | X | | X | X |
| *Pseudomonas* | X | X | X | X |

[a] With permission from Klein and Thayer (1990).

*Alcaligenes*, and *Acinetobacter* might also be included based on Hg resistance, which correlates with $Hg^{2+}$-reducing ability.

### Biomethylation Transformations

Microorganisms have been shown to be intimately involved in the interconversions of metals between inorganic and organic compounds. Many metals and metalloids with toxic potential, such as Hg, As, Cd, Pb, Se, Sn, and Te, are subject to alkylation. Binding a C atom to the metal changes an element's volatility, solubility, and toxicity. All biotic alkylations are accomplished by one of three coenzymes: *N*-methyltetrahydrofolate, *S*-adenosylmethionine, or methylcobalamine. Methylation is accomplished by a wide array of fungi and bacteria (Table 15.13), and a given species of microorganism may methylate several different metals. Methylation affects elements differently. Cadmium alkyls are water insoluble, while the methylated forms of As are soluble and highly toxic. Similarly to Hg, methylated Se undergoes atmospheric transformations that foster detoxification, deposition, and bioaccumulation.

The methylation of Hg can occur under aerobic or anaerobic conditions, but the process was found to occur more rapidly under the latter conditions (Robinson and Tuovinen, 1984). Aerobic organisms such as *Pseudomonas* spp., *Bacillus megaterium*, *E. coli*, *En. aerogenes*, and even fungi such as *A. niger* and *Scopulariopsis brevicaulis* have been found capable of Hg methylation. However, sulfate reducers such as *Desulfovibrio desulfuricans* appear to be the principal methylators of Hg. The reaction occurs through the transfer of methyl groups from methylcobalamine (cobalt prophyrin), or from methylated vitamin $B_{12}$, to $Hg^{2+}$. The normal role of methylcobalamine in *D. desulfuricans* is to provide the methyl group in

acetate synthesis from $CO_2$ using the acetyl-SCoA pathway. The $Hg^{2+}$ acts as a competing methyl acceptor during acetate formation (Choi *et al.*, 1994). The microbially mediated reaction is a protection mechanism for the microorganism as it increases the solubility and volatility of the Hg. However, methylmercury is lipophilic and can be accumulated in higher organisms.

## ENVIRONMENTAL SIGNIFICANCE OF P, S, AND METAL BIOGEOCHEMISTRY

### EUTROPHICATION

Phosphorus is the most limiting nutrient for primary production in many ecosystems, particularly aquatic systems. However, P can cause environmental damage if it is applied in excess. Excess P in soil may move in soluble form in runoff water or attached to eroded soil particles to receiving surface waters. Eutrophication is an increase in the fertility status of natural waters that causes accelerated growth of algae or water plants. It is not caused by inputs of P alone, but by a complex interaction between N, P, environmental conditions such as light, and the hydrologic characteristics of surface waters. Since P limitations are common in many receiving waters, the addition of P is the most direct factor in increased eutrophication (Correll, 1998). As P input to surface waters increases, the trophic state of the water body goes from oligotrophic (low productivity), through mesotrophic, to eutrophic (high primary productivity). Once eutrophic conditions are established dissolved $O_2$ concentrations will decrease, turbidity will increase, and excessive water plant growth and algal blooms will become more frequent. The increased input of P permits increases in algal biomass, which is visible in eutrophic lakes or ponds as a green surface scum. When the algae die, they settle to the bottom and become substrate for heterotrophic benthic organisms. The decomposition of the dead algae results in oxygen depletion. In extreme cases, eutrophication may cause severe ecological changes such as fish kills due to the oxygen depletion and cause impacts on recreational and navigational uses.

### ACID SULFATE SOILS

Acid sulfate soils have been described as the “nastiest soils in the world” (Dent and Pons, 1995). They contain high concentrations of sulfide, mainly in the form of pyrite ($FeS_2$), which may be oxidized to yield free and adsorbed sulfates, resulting in extremely acid soils (pH’s as low as 2). Dent and Pons (1995) have described the pedologic formation of these soils. In the first step, bacteria decomposing the abundant organic matter in tidal swamps and marshes reduce $SO_4^{2-}$ from the tidewater and $Fe^{3+}$ oxides from the sediment. The main end product is pyrite, which remains reduced in the tidal zone and when buried by peat or freshwater alluvium. Extensive areas of unripe sulfidic clays are still found in Indonesia, West Africa,

and northern Australia. Some sulfidic sediments have been drained naturally by tectonic uplift or through changes in the hydrology of deltas. However, there has been an incentive to reclaim swamplands in an attempt to exploit the expected fertility. Problems arise whenever the rate of acid production from oxidation of sulfides exceeds the buffering capacity of the soil. Acid sulfate soils produced by drainage become severely acid within weeks or months. Usually, they can be identified by straw yellow mottles of jarosite, $KFe_3(SO_4)_2(OH)_6$, that develop on ped surfaces and by acidic, red drainage water. The major limitation to soil productivity in many cases is not the acidity of the soil itself, but increased aluminum and manganese toxicity to plants caused by the release of these elements due to the acidification.

The acidification of these soils results from the abiotic and microbial oxidation of pyrite when reducing conditions no longer exist. The overall reaction is

$$3\ FeS_2 + 11\ O_2 + 2\ H_2O \rightarrow 3\ Fe^{3+} + 4\ H^+ + 6\ SO_4^{2-}.$$

This summarizing equation is somewhat misleading in that the primary oxidant involved in pyrite oxidation in most situations is ferric iron rather than molecular oxygen, and pyrite oxidation is a multistep process involving an oxygen-independent reaction and oxygen-dependent reactions. The proper sequence is

$$2\ FeS_2 + 7\ O_2 + 2\ H_2O \rightarrow 2\ Fe^{2+} + 4\ H^+ + 4\ SO_4^{2-},$$

$$2\ Fe^{2+} + O_2 + 4\ H^+ \rightarrow 2\ Fe^{3+} + 2\ H_2O,$$

$$2\ Fe^{3+} + FeS_2 \rightarrow 3\ Fe^{2+} + 2\ S^0,$$

$$2\ S^0 + 3\ O_2 + 2\ H_2O \rightarrow 4\ H^+ + 2\ SO_4^{2-}.$$

The second step (e.g., the regeneration of ferric iron, which is then reduced to ferrous on reaction with pyrite) is the key reaction in promoting the ongoing oxidation of the mineral. At pH values $>4$, this may be mediated chemically or biologically (by iron-oxidizing bacteria such as *Ga. ferruginea*), while at pH $< 4$, abiotic oxidation is negligible and the activities acidophilic iron-oxidizing bacteria dominate (Johnson and Hallberg, 2003).

## ACID MINE DRAINAGE

Many ores of metals such as Cu, Zn, and Pb contain pyritic materials. In addition, high-S coals also contain substantial amounts of pyrite and organic S. When the ores or coal are removed, the traditional practice has been to leave the mine spoils in heaps of tailings exposed to air and water. While sulfide material oxidation may be abiotic, the reaction rate is orders of magnitude greater in the presence of oxidizing bacteria such as *Ac. ferrooxidans.* The result is the accelerated oxidation of pyrite and other sulfidic minerals in the same process described above for acid sulfate soils. Acid mine drainage (AMD) includes the acidified water that percolates through aboveground mine tailings and to a lesser extent the groundwater that

becomes contaminated when underground mine workings are abandoned and the water table is allowed to rebound. Uncontrolled leaching of mine tailings and abandoned sites is a major cause of environmental degradation and expensive remediation in numerous areas. In the United States alone, AMD from abandoned mine sites has polluted 75,000 ha of impoundments and lakes, as well as 20,000 km of rivers and streams (Pierzynski *et al.*, 2000), and it continues to be the most important environmental challenge for the mining industry.

Acid mine drainage environments are scientifically interesting as model ecosystems for the analysis of biogeochemical interactions and feedbacks and microbial community structure and function (Baker and Banfield, 2003, Fig. 15.11). Microbial communities in AMD systems tend to contain few distinct taxa, but these taxa are phylogenetically diverse. In regions of AMD systems exposed to sunlight, photosynthesis is an important source of energy; however, below the surface, inputs of externally derived fixed C and N are minimal. The primary metabolic groups detected in AMD systems are lithoautotrophs that oxidize $Fe^{2+}$ and $S^-$ released by pyrite dissolution, organoheterotrophs that utilize C produced by the lithoautotrophs, lithoheterotrophs that oxidize Fe and S, and anaerobes that couple oxidation of S or organic C to $Fe^{3+}$ reduction. A subset of these organisms must produce all the fixed C and N required by the community. At lower temperatures (<30ºC) and higher pH (>2), the thiobacilli are probably the dominant group responsible for $CO_2$ fixation. At lower pH and higher temperatures, autotrophic taxa include *Leptospirillum* spp., *Ferroplasma* spp., *Sulfobacillus* spp., *Ferromicrobium* spp., and *Acidimicrobium* spp. The supply of N to the system is more problematic because in the largely aerobic AMD environment, $N_2$ fixation by

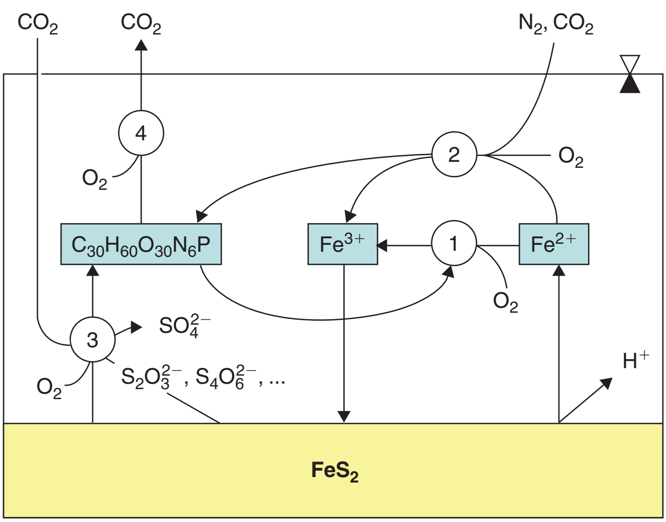



FIGURE 15.11 A simplified acid mine drainage system showing examples of organisms (1, 2, 3, 4) that control the flow of iron, sulfur, nitrogen, carbon, and energy in different temperature regimes (with permission from Baker and Banfield, 2003).

nitrogenase is inhibited. In moderately acidic AMD, *Ac. ferrooxidans* may overcome this problem by using tetrathionate as an electron donor and ferric iron (rather than $O_2$) as an electron acceptor when fixing N. N fixation has not yet been observed directly in very low pH environments. Another important symbiosis exists between heterotrophic and certain autotrophic species in the AMD ecosystem: autotrophs may depend on coexisting heterotrophs to remove organic compounds that are toxic to them.

Given that both oxygen and water are required for the formation of AMD, it should be possible to prevent or minimize AMD production by excluding one, or both, of these (Johnson and Hallberg, 2005). Options for preventing the formation of AMD include: (1) flooding or sealing of underground mines, (2) underwater storage of mine tailings, (3) land-based storage of sealed tailings, (4) blending of mineral wastes, (5) total solidification of tailings, (6) application of anion surfactants, and (7) microencapsulation (coating) of wastes. Given the practical difficulties involved in inhibiting the formation of AMD at the source, the strategy more often applied is to minimize the impact that AMD has on receiving streams and rivers using "migration control" measures. These measures have been divided between those remediation technologies that rely on biological activities and those that do not. Abiotic remediation strategies generally involve the addition of alkaline materials such as lime to neutralize the pH of the AMD, causing many of the metals in solution to precipitate. The basis of biological remediation of AMD relies on the abilities of some microorganisms to generate alkalinity and immobilize metals. Microbiological processes that generate net alkalinity are mostly reductive processes and include methanogenesis, sulfate reduction, and iron and manganese reduction. The current state of the art of bioremediation of AMD is to exploit these reactions in constructed aerobic wetlands, compost reactors, or packed-bed iron oxidation bioreactors.

## HEAVY METAL MINING USING MICROBES

The use of microorganisms to extract metals from ore involves the harnessing of a natural process in an industrial context. Biomining (a general term referring to microbially enhanced leaching or oxidation of metals) has some distinct advantages over traditional methods, in that it is more environmentally friendly. It does not have the high energy requirements of smelting and does not produce harmful gaseous emissions. The wastes produced from biomining operations are less biologically and chemically active than traditional mine tailings because it has already been bioleached. Microbial leaching of ore bodies *in situ*, in stockpiles, or in mine tailings also makes metal recovery feasible from low-grade ores in which the metal is not economically recoverable by smelting. For example, biooxidation of refractory gold-bearing ores allows this low-grade ore with as little as 1 g of gold (Au) per metric ton, which would otherwise be considered as waste, to be processed at a cost in range of U.S. $4–6 per metric ton (Rawlings, 2002). Biomining is commonly used for recovery of Au and uranium (U), but copper (Cu) is recovered

in by far the greatest quantities. Ferric sulfides usually accompany the Cu sulfides in ore and hence Fe transformations are components of the reaction chain. Ferrous iron is formed in the ore body by reaction of ferric iron with CuS:

$$2\,Fe^{3+} + CuS \rightarrow Cu + 2\,Fe^{2+} + S^0.$$

The mobilized Cu is recovered from the leachate by sedimentation, solvent action, or electrolysis. The $Fe^{2+}$ remaining after Cu removal is reoxidized (see the previous equation). Also, sulfur-oxidizing bacteria convert $S^0$ to sulfuric acid:

$$S^0 + H_2O + \tfrac{3}{2}\,O_2 \rightarrow H_2SO_4.$$

This acid fortifies the leaching solution, which is recirculated following the metal harvest.

The organisms involved in biomining are acidolithic, chemolithoautotrophic, able to use ferrous iron or reduced S or both as electron donors, and able to fix $CO_2$. Among important species in ore leaching are the following: *Acidithiobacillus ferrooxidans*, *T. thiooxidans*, *Leptospirillum ferrooxidans*, *Sulfobacillus thermosulfidooxidans*, and *Acidianus brierleyi.* A properly aerated ore body or suspension of iron- and S-containing mineral in water is an ideal environment for these organisms: air provides the C source ($CO_2$) and preferred electron acceptor ($O_2$), the mineral ores supply the electron donors, and water is the medium for growth. Inoculation of the ore body with microorganisms is usually unnecessary. Normally occurring Fe and S oxidizers and acidophilic heterotrophs establish themselves unaided because the acidic environment is not suitable for the growth of other organisms.

Several commercial-scale biomining processors are operating around the world (Rawlings, 2002). The two main types are irrigation-type and stirred-tank processes. Irrigation processes involve the percolation of leaching solutions through crushed ore that has been stacked in heaps or dumps or irrigation of an ore body *in situ* without bringing the ore to the surface. Stirred-tank-type processors are large (1000–2000 $m^3$) tanks arranged in series and operated in continuous flow mode, in which the feed being added to the first tank overflows from tank to tank until the process is sufficiently complete. The stirred-tank operations provide a step up in rate and efficiency of biooxidation, but are expensive to construct and operate. Their use is therefore restricted to the pretreatment of high-value gold-bearing arsenopyrite concentrate. Most current biomining processes are operated at ambient temperatures or in tanks at 40–50ºC. Warmer operations are currently being developed, which will accelerate the process and make it more economical, but will also require a better understanding of the thermophilic organisms involved.

## MICROBIAL CORROSION OF BURIED IRON AND CONCRETE PIPES

Most microbially influenced corrosion occurs in the presence of microbial consortia in which many different physiological types of bacteria interact within the structure of biofilms. In the anaerobic corrosion of buried iron pipes, the metal

TABLE 15.14 Some Bacteria Involved in Corrosion Processes[a]

| Organism | Oxygen requirement | Inorganic component | Metabolic end products | Habitat | Optimal range: Temp. (°C) | Optimal range: pH |
|---|---|---|---|---|---|---|
| Sulfate reducing: *Desulfovibrio desulfuricans* | Anaerobic | Sulfate, thiosulfate | Hydrogen sulfide | Water, soil, mud, oil reservoir | 25–30 | 6–7.5 |
| Sulfur oxidizing: *Acidithiobacillus thiooxidans* | Aerobic | Sulfur, thiosulfate | Sulfuric acid | Soil, water | 28–30 | 2–4 |
| Thiosulfate oxidizing: *Thiobacillus thioparus* | Aerobic | Thiosulfate, sulfur | Sulfur, sulfuric acid | Soil, water, mud, sewage | 28–30 | 7 |
| Iron bacteria: *Crenothrix*, *Leptothrix*, *Gallionella* | Aerobic | Iron, manganese | Ferric or manganese oxides | Water | 25 | 8 |
| Nitrate reducing: *Thiobacillus denitrificans* | Facultative | Thiosulfate, sulfur, sulfide | Sulfate | Soil, mud, peat, water | 30 | 7–9 |
| Hydrogen utilizing: *Hydrogenomonas* | Microaerophilic | Hydrogen | Water | Soil, water | 28–30 | 7 |

[a]With permission from Atlas (1984).

surface acts as an anode in an electrochemical reaction and is transformed to $Fe^{2+}$. An equivalent number of $H^+$ ions are produced at the cathode site. The anaerobic, sulfate-reducing bacteria such as *Desulfobacter* and *Desulfovibrio* produce $S^{2-}$, which reacts with $Fe^{2+}$ to produce FeS. At the same time, hydroxyls from water react with $H^+$ ions. The overall reaction is

$$4\ Fe + SO_4^{2-} + 4\ H_2O \rightarrow FeS + 3\ FeOH^{2-} + 5\ OH^-.$$

The conditions required for the reaction include anaerobic sites at redox potentials less than −400 mV, a pH greater than 5.5, low free-$O_2$ content, and the presence of $SO_4^{2-}$. Under these conditions, an Fe pipe of 3-mm wall thickness can be corroded through in 5 to 7 years. This is one of the most costly microbial reactions in nature. It means that buried Fe pipes must be continually replaced or else protected by wrapping with asphalt or plastic. Additional protection is obtained by maintaining a small electrical current along the pipe (cathodic protection) to prevent the formation of an electrode half-cell.

Other corrosion processes are accomplished by miscellaneous aerobic and facultative bacteria (Table 15.14). Metal pipes are not the only ones to be affected by microbially induced corrosion. Concrete is a moderately porous mixture of alkaline precipitates and mineral aggregate. Strong acids react with the alkaline materials, causing structural degradation. Concrete sewer pipes are subject to corrosion by a mutualism between autotrophs and heterotrophs and succession of acidophiles (Little *et al.*, 2000; Fig. 15.12).

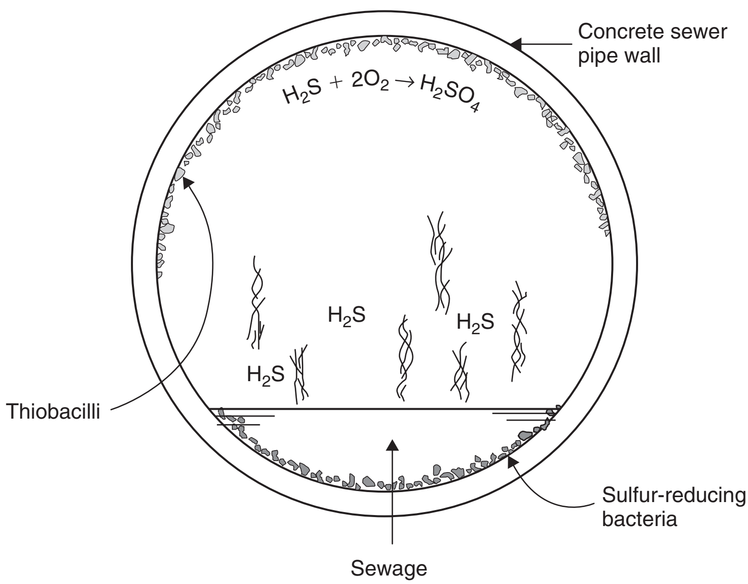



FIGURE 15.12 Conceptual diagram of the mutualistic relationship between sulfur-reducing bacteria and thiobacilli that causes corrosion in concrete sewer pipes (with permission from Little *et al.*, 2000).

Anaerobic conditions in the sewage support sulfur-reducing bacteria that convert sulfate and organic S to $H_2S$, which volatilizes to the sewer atmosphere. The $H_2S$ redissolves in condensate upon reaching the sewer crown, where a second community of aerobic microorganisms including thiobacilli oxidizes it to corrosive $H_2SO_4$. The organism involved in the latter step was called *Thiobacillus concretivorous* when isolated, but was found to be *Acidithiobacillus thiooxidans*.

## CONCLUSION: MICROORGANISMS AS UNIFIERS OF ELEMENTAL CYCLES IN SOIL

Soil microorganisms not only have a profound influence on the fluxes and cycling of individual element cycles, but also are strong integrators of the various element cycles. They integrate C, N, S, P, and various metal element cycles through biomass production in two related ways: (1) the stoichiometry of biomass production and (2) the selection of alternative electron acceptors under different redox conditions.

Stoichiometric relationships between substrates and decomposers may provide a framework to understanding how the internal cycling of nutrient elements in soil is controlled. It would follow that the release of inorganic forms of N, S, and P would occur following the oxidation of C to $CO_2$ in ratios similar to those present in the organic component from which the C was derived. The frequent reporting of average nutrient concentrations and stoichiometric ratios of organisms and soil (e.g., Table 15.3) suggests that these values are reasonably constant and the approach sound. However, the stabilization of organic materials containing N, S, and P in soil is strongly influenced by the soil matrix itself through various physical and chemical protection mechanisms. The protection afforded by the inorganic matrix is a distinguishing feature of the soil environment compared to aquatic systems. Consequently, while biomass stoichiometry may be a dominant control on nutrient cycling in aquatic systems (e.g., Redfield, 1958), its potential control is shared with overriding protection mechanisms in terrestrial systems.

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# 16

# THE DYNAMICS OF SOIL ORGANIC MATTER AND NUTRIENT CYCLING

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WILLIAM J. PARTON



## INTRODUCTION

Knowledge of the turnover rates of plant and animal residues, microbial bodies, and soil organic matter (SOM) is a prerequisite for understanding the availability and cycling of nutrients such as C, N, S, and P. This understanding is essential in describing ecosystem dynamics and in calculating crop nutrient needs relative to environmental pollution control. It is also necessary if we are to gain an understanding of how turnover might affect C sequestration and, in turn, be altered by global change. The significance of microbially mediated decay products involved in SOM decomposition and nutrient cycling can best be determined using mathematical analysis of tracer and non-tracer data. This requires knowledge of the various reservoirs or pool sizes in the system under study and the rates at which materials are transformed within and transferred between them.

## REACTION KINETICS

Understanding the dynamics of nutrient, plant residue, or SOM transformations in the field requires meaningful mathematical expressions for the biological, chemical, and physical processes involved. The reaction rate of decomposition (represented by the change in substrate concentration with time, *dS/dt*) can be expressed as a function of the concentration of one or more of the substrates being degraded. The order of the reaction is the value of the exponent on the substrate concentration in the equation used to describe the reaction.

### ZERO-ORDER REACTIONS

Zero-order reactions are those in which the rate of transformation of a substrate is unaffected by changes in the substrate concentration (the exponent on $S$ on the right-hand side of the equation is zero, $S^0 = 1$, thus zero order). Zero-order kinetics can be described using the following equation

$$\frac{dS}{dt} = -k \tag{1}$$

In this case, the reaction rate is determined by factors other than the substrate concentration, such as the amount of catalyst. At high substrate concentrations, at which substrate levels are not limiting, enzymatic reactions are usually zero order, e.g., nitrification at high $NH_4^+$ levels and denitrification at high $NO_3^-$ levels. Figure 16.1 shows plots of zero-order reactions compared to plots of other kinetic equations. After integration, the equation can be solved for the substrate concentration as a function of time and becomes

$$S_t = S_0 - kt \tag{2}$$

where $S_t$ (concentration) is the amount of substrate remaining at any time, $S_0$ (concentration) the initial amount of substrate in the system, $k$ (concentration time$^{-1}$) the rate constant, and $t$ (time) the time since the initiation of the reaction. A useful term to describe the reaction kinetics is the half-life, which is the time required to transform one-half of the initial substrate, e.g.,

$$S_t = S_0/2 \text{ then}$$

$$t_{1/2} = S_0/2k \tag{3}$$

The mean residence time (or turnover time) is the time required to transform a quantity of material equal to the starting amount $S_0$ (i.e., $S_t = S_0$) then at steady state

$$t_{\mathrm{mrt}} = S_0/k \tag{4}$$

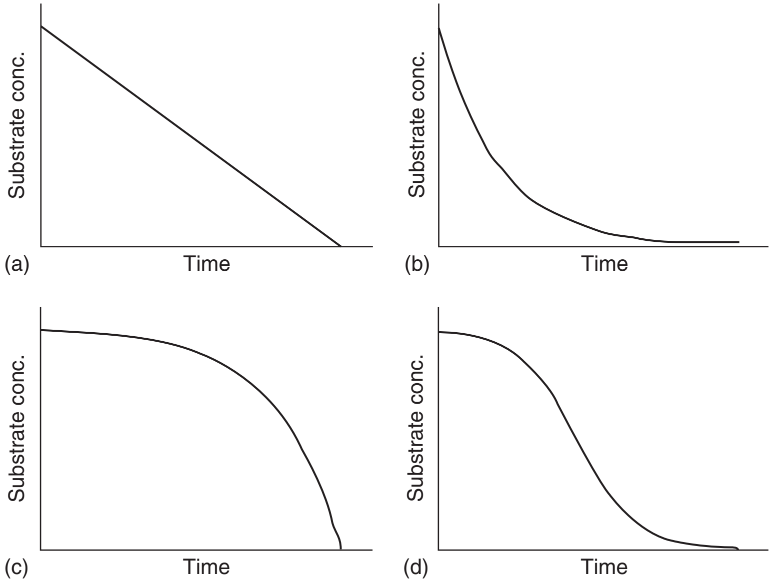



FIGURE 16.1 Graphical representation of kinetic equations depicting the shapes of substrate decomposition curves: (a) zero order, (b) first order, (c) with exponential growth, and (d) with logistic growth.

## FIRST-ORDER REACTIONS

In first-order reactions, the rate of transformation of a substrate is proportional to the substrate concentration. The rate of change of substrate $S$ with time is

$$\frac{dS}{dt} = -kS \tag{5}$$

The decrease in the concentration of the substrate with time $t$ is dependent on the rate constant $k$ times the concurrent concentration $S$ of the substrate. After integration, we obtain the equation

$$S_t = S_0 e^{-kt} \tag{6}$$

where $S_t$ is the concentration of the substrate remaining at any time $t$. The rate constant $k$ involves some expression per unit time (e.g., $h^{-1}$, $day^{-1}$, or $year^{-1}$).

To calculate the time required to transform one-half the initial substrate ($S_t = S_0/2$), we start with

$$\ln\left[\frac{(S_0/2)}{S_0}\right] = -kt_{1/2}$$

which is

$$t_{1/2} = \ln 2/k \approx 0.693/k \tag{7}$$

The mean residence time (turnover time of an amount of substrate at steady state equivalent in size to the starting amount) for first-order reactions is equal to $1/k$. Note that $t_{mrt}$ for zero-order reactions is $S_0/k$.

## ENZYMATIC KINETICS

Because extracellular enzymes are responsible for much of the substrate depletion in soils, the kinetics of enzyme reactions can be used to model substrate depletion. Enzyme kinetics are represented by the hyperbolic, Michaelis–Menton equation,

$$\frac{dS}{dt} = V_m \frac{S}{K_m + S} \tag{8}$$

where $V_m$ is the maximum reaction rate (concentration time$^{-1}$), and is proportional to the total mass of enzyme in the soil (and hence to the total active biomass), and $K_m$ is the Michaelis–Menton, or half-saturation, constant and is the substrate concentration at which the reaction occurs at half the maximum velocity, $V_m/2$. $K_m$ is inversely related to enzyme–substrate affinity, which tends to have higher values in soil than in aqueous solutions and to decrease when soil slurries are shaken; therefore $K_m$ is inversely proportional to diffusivity.

Figure 16.2 illustrates how Michaelis–Menton kinetics contain both first- and zero-order regions.

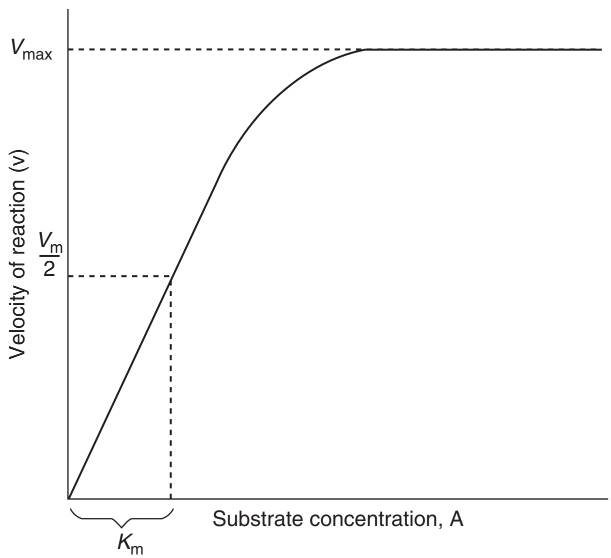



FIGURE 16.2 Graphical expression of the Michaelis–Menton kinetic parameters for an enzymatic reaction.

At high substrate concentrations ($S \gg K_m$), the equation simplifies to

$$\frac{dS}{dt} = V_m \tag{9}$$

which describes a zero-order reaction, and under very low substrate concentrations ($S \ll K_m$), the equation simplifies to

$$\frac{dS}{dt} = \frac{V_m S}{K_m} = k'S \tag{10}$$

which describes a first-order reaction. Because of the combined linear and exponential forms of $S$ in the equation, the Michaelis–Menton cannot be solved for $S_t$ analytically; however, solutions are easily calculated iteratively using spreadsheet software.

## MICROBIAL GROWTH

The equations described above have one limitation in common; none can account for microbial growth. As microorganisms consume a substrate, one portion is used for maintenance energy requirements and if enough substrate is available, the remainder will be used to support growth. As microorganisms grow and multiply they will exert an increasing demand on the remaining substrate, thereby changing the kinetics of decomposition. Three equations can be used to describe microbial growth: the exponential, logistic, and Monod equations.

When rapidly consuming substrate, microbes are known to grow exponentially,

$$\frac{dN}{dt} = \mu N \tag{11}$$

where $N$ is the number (biomass) of cells and $\mu$ is the growth rate. Changes in biomass can be equated to changes in substrate by dividing the biomass by the yield ($y$), which is the mass of cells generated per mass of substrate consumed:

$$-\frac{dS}{dt} = \frac{dN}{dt}\frac{1}{y} \tag{12}$$

Substituting Eq. (11) for $dN/dt$ into Eq. (12), we obtain the following equation in terms of substrate depletion,

$$-\frac{dS}{dt} = \mu\frac{N_t}{y} \tag{13}$$

where $N_t$ is the biomass at any given time. Measuring biomass at all given times is unrealistic, therefore $N_t$ can be described in terms of initial biomass, $N_0$, such that $N_t = N_0 + N_t - N_0$, and the above equation expands to

$$-\frac{dS}{dt} = \mu\frac{N_0}{y} + \mu\frac{N_t}{y} - \mu\frac{N_0}{y}$$

and collecting terms

$$-\frac{dS}{dt} = \mu\frac{N_0}{y} + \mu\frac{(N_t - N_0)}{y} \tag{14}$$

Expressing all terms as substrate, considering that the total amount of substrate consumed since $t = 0$ is $S_0 - S_t = (N_t - N_0)/y$, and letting $X_0 = N_0/y$ where $X_0$ becomes the amount of substrate needed to produce $N_0$, then the differential form of the exponential equation for substrate depletion is

$$-\frac{dS}{dt} = \mu(S_0 + X_0 - S) \tag{15}$$

While the exponential equation links substrate depletion with microbial growth, it is well recognized that microbes do not grow exponentially at all times. Rather, microbial populations grow to a limit ($K$). As the population increases, the growth rate ($\mu$) decreases due to competition among individuals for increasingly scarce substrate resources. Microbial growth to a limit is expressed by the logistic equation

$$\frac{dN}{dt} = \mu\left(1 - \frac{N}{K}\right)N \tag{16}$$

A similar exercise of algebra to express the logistic equation in terms of substrate, considering that $K$ is the maximum biomass (original biomass plus that generated by converting all the original substrate into biomass), yields the differential equation for logistic growth in terms of substrate depletion:

$$-\frac{dS}{dt} = \mu\left(\frac{S}{S_0 + X_0}\right)(S_0 + X_0 - S) \tag{17}$$

Initially, $S = S_0$ and the reaction rate is governed primarily by $X_0$. As the microbial population grows, $S$ decreases such that the first term in the equation above decreases while the second term increases. Therefore, two competing trends govern the substrate depletion rate. The rate of substrate depletion is maximized at $-dS/dt_{max} = \mu(S_0 + X_0)/4$. The differential form of the logistic equation can be integrated and solved for $S_t$ to give

$$S_t = S_0 + X_0 - \frac{S_0 + X_0}{1 + \left(\frac{S_0}{X_0} - 1\right)e^{-\mu t}} \tag{18}$$

Monod kinetics are the most general kinetic expressions because they relate substrate depletion both to changes in population density and to changes in substrate concentration. The basic relationship is

$$\mu' = \mu_{\max} \frac{S}{K_s + S} \tag{19}$$

which appears similar to the Michaelis–Menton equation but has some subtle differences: $\mu'$ is the specific growth rate ($\mu' = \mu/N_t$), $\mu_{\max}$ is the maximum growth rate when substrate is not limiting, and $K_s$ is the Monod constant, which is similar to the Michaelis–Menton constant. The differential equation describing Monod kinetics with growth in terms of substrate depletion is

$$-\frac{dS}{dt} = \mu_{\max} \frac{S}{K_s + S} (S_0 + X_0 - S) \tag{20}$$

As with the Michaelis–Menton equation, the integrated form of Monod kinetics cannot be solved for $S_t$, but can be solved for $t$, and then the substrate concentration can be determined iteratively.

While apparently complex, Monod kinetics can be simplified under certain conditions to yield each of the kinetic equations described above. Table 16.1 summarizes the various kinetic equations and the conditions under which they will occur. For instance, when the initial concentration of microbial biomass is much greater than the initial substrate concentration (e.g., $X_0 \gg S_0$), then the term $(S_0 + X_0 - S)$ in the Monod equation can be approximated to $X_0$ and the Monod equation is simplified to the Michaelis–Menton equation used to describe enzyme kinetics.

## MODELING THE DYNAMICS OF DECOMPOSITION AND NUTRIENT TRANSFORMATIONS

Models can be used to gain an understanding of the processes and controls involved in nutrient cycles, to generate data on the size of various pools and the rates at which nutrients are transformed, and to make predictions when experiments are inappropriate. While conceptual models may be sufficient for the first task, only quantitative models can achieve the latter tasks. Quantitative models of SOM and nutrient dynamics are attempts to describe soil biological processes rather than strictly mathematical expressions and statistical procedures used to find best-fitting curves. Fitting model equations to carbon and nutrient mineralization curves provides estimates of the amount of mineralized product released (e.g., $CO_2$) and the rate at which the product (e.g., $NO_3^-$) is made available to plants. Models range from single-equation kinetic representations such as those outlined above to large mechanistic models that account for many components of an ecosystem and require computers for generating the results.

**TABLE 16.1** The Monod Kinetic Equation, Its Simplifications, and the Conditions under Which the Simplifications Can Be Made

| Condition | Outcome | Kinetics | Differential form | Integrated form | Solve for $S$? |
|---|---|---|---|---|---|
| | | Monod | $-\frac{dS}{dt} = \mu_{max} \frac{S}{K_s + S}(S_0 + X_0 - S)$ | $K_s \ln\left(\frac{S_t}{S_0}\right) = (S_0 + X_0 + K_s)\ln\left(\frac{X_t}{X_0}\right) - (S_0 + X_0)\mu_{max} t$ | N |
| $S_0 \gg K_s$ | $K_s + S \approx S$ | Exponential | $-\frac{dS}{dt} = \mu_{max}(S_0 + X_0 - S)$ | $S_t = S_0 + X_0 - X_0 e^{kt}$ | Y |
| $S_0 \ll K_s$ | $K_s + S \approx K_s$ | Logistic | $-\frac{dS}{dt} = \frac{\mu_{max} S}{K_s}(S_0 + X_0 - S)$ | $S_t = S_0 + X_0 - \frac{S_0 + X_0}{1 + \left(\frac{S_0}{X_0} - 1\right)e^{-\mu t}}$ | Y |
| $X_0 \gg S_0$ | $S_0 + X_0 - S \approx X_0$ | Michaelis–Menton | $-\frac{dS}{dt} = \mu_{max} \frac{S X_0}{K_s + S} = k' \frac{S}{K_s + S}$ | $S_0 - S_t + K_s \ln\left(\frac{S_0}{S_t}\right) = V_m t$ | N |
| $X_0 \gg S_0$ and $S_0 \ll K_s$ | $S_0 + X_0 - S \approx X_0$ $K_s + S \approx K_s$ | First order | $-\frac{dS}{dt} = \frac{\mu_{max} S X_0}{K_s} k' S$ | $S_t = S_0 e^{-kt}$ | Y |
| $X_0 \gg S_0$ and $S_0 \gg K_s$ | $S_0 + X_0 - S \approx X_0$ $K_s + S \approx S$ | Zero order | $-\frac{dS}{dt} = \mu_{max} X_0 = k'$ | $S_t = S_0 - kt$ | Y |

## SIMPLE MODELS

The kinetics of plant nutrient transformations has been of interest for a long time, particularly the kinetics of N mineralization. Stanford and Smith (1972) began by describing net N mineralization using a simple first-order model, $N = N_0(1 - e^{-kt})$, where $N$ is the amount of nitrogen mineralized at time $t$ and $N_0$ is the amount of potentially mineralizable nitrogen. Several modifications to first-order models, as well as other kinetic models, have been proposed to account for experimental observations of large initial flushes of mineralization or for lags before the initiation of mineralization (Ellert and Bettany, 1988). In selecting a model for N and S net mineralization, one should generally increase model complexity incrementally to obtain a suitable fit, keep the number of parameters to a minimum, and know that no single model will fit data for all soils under all conditions, while some conditions will be adequately described by several models.

Because they play different roles in plants and the environment that lead to differing dynamics, there are few short-term or single-season models for C like there are for N and other plant nutrients. Soil organic matter models generally project the long-term sustainability of changes in soil C, but there are a number of ways to describe the short-term decomposition of organic residues during the first few months after introduction to the soil. Field and laboratory experiments have shown that initial decomposition rates of litter are generally independent of the amount of biomass added unless it exceeds 1.5% of the dry soil weight. Decomposition of plant residues has been experimentally found to be reasonably well described by first-order rate kinetics. The use of first-order kinetics to describe the decomposition of SOM implies that the microbial inoculum potential of soil is not limiting the decomposition rate (e.g., $X_0 \gg S_0$, from the previous section). This is true, in large part, because soil microbial biomass often has a fast growth rate relative to the length of most decomposition studies.

Jenny (1941) published a simple model that used a combination of zero-order and first-order components to describe changes in soil organic matter,

$$\frac{dX}{dt} = A - kX$$

where $X$ is the organic C or N content of the soil and $A$ is the addition rate (mass $t^{-1}$) used to describe accumulations or losses not associated with decomposition. However, this model does not account for the heterogeneous nature of SOM, i.e., $k$ is constant. Several approaches have been used to accommodate for the changing nature of organic matter during decomposition, such as making $k$ a function of time or including additional compartments.

Experimental data for the decomposition of added plant residues or manures can be closely fit using the summation of two first-order equations in the general form

$$C = Ae^{-k_A t} + Be^{-k_B t}$$

where $C$ is the soil C content at any given time, $A$ and $B$ are the proportions of the two pools, and $k_A$ and $k_B$ are the first-order constants for each of the pools. In the case

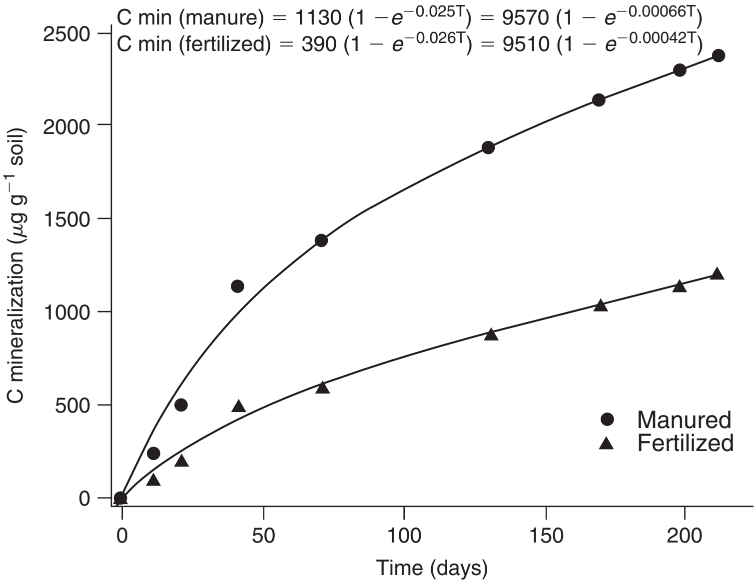



FIGURE 16.3 The fit of the sum of two first-order curves describing C mineralization in soil from a manured and fertilized long-term plot during a 220-day incubation.

illustrated in Fig. 16.3, the first pool of the manured treatment represented 5% of the soil C and had a turnover time of 40 days in the laboratory. The second pool represented 45% of the C with a laboratory turnover time of 3 years. The curve representing the fertilized plots showed that the first pool represented 2% of the C with a turnover time similar to that of the manured (40 days). The second pool representing 48% of the C had a turnover time of 5 years. The remainder of the C was known from $^{14}C$ dating to have a turnover time of 500 to 1000 years and therefore did not contribute $CO_2$ to the decomposition of the inputs. The example demonstrates how the partitioning of organic matter between labile and more resistant fractions alters the decomposition dynamics. Lack of participation of a significant proportion of the soil C in respiration suggests the need for an additional pool or compartment, and this knowledge led to the development of the multicompartmental models.

Differences in the ability of simple models to model short-term versus long-term decomposition dynamics were highlighted by Sleutel *et al.* (2005), who compared the performance of first-order, sum of first-order, combination of zero-order and first-order, second-order, and Monod kinetic models for extrapolation from short-term data. They concluded that the sum of first-order and Monod models performed best in estimating stable organic C, but did not fit short-term mineralization well, while the first-order and combination of zero-order and first-order models should not be used for extrapolating from short-term data.

Only a portion of the actual decomposition is accounted for when determining the decomposition rate ($k$) by measuring $CO_2$ output or the amount of C left in the soil.

TABLE 16.2 First-Order Decay Constants with and without Correction for Microbial Biosynthesis during the Decomposition of Organic Compounds Added to Soil under Laboratory Conditions

| | | $k$ (day$^{-1}$) | | |
|---|---|---|---|---|
| Material | Time of incubation (days) | Uncorrected | Corrected for CUE = 20% | Corrected for CUE = 60% |
| Straw–rye | 14 | 0.02 | 0.03 | 0.11 |
| Hemicellulose | 14 | 0.03 | 0.04 | 0.11 |
| Lignin | 365 | 0.003 | 0.006 | — |
| Native grass | 30 | 0.006 | 0.008 | 0.02 |
| Fungal cytoplasm | 10 | 0.04 | 0.05 | 0.17 |
| Fungal cell wall | 10 | 0.02 | 0.03 | 0.07 |

Microorganisms use C compounds for biosynthesis, forming new cellular or extracellular material, and as an energy supply. In the latter process, $CO_2$, microbial cells, and waste products are produced. Under aerobic conditions, the amount of waste products produced is not usually high, and the amount of biosynthesis, or production of microbial cells, can be calculated from $CO_2$ data. This requires knowledge of yield or efficiency of substrate conversion to microbial biomass,

$$C = C_i[1 + Y/(100 - Y)]$$

where $C$ is the substrate decomposed, $C_i$ the $CO_2$–C evolved, and $Y$ the efficiency (yield, or sometimes CUE for C utilization efficiency) of the use of $C$ for biosynthesis, expressed as a percentage of the total $C$ utilized for production of microbial material. The decomposition rate constants ($k$), corrected for biosynthesis, differ significantly from the uncorrected ones (Table 16.2). Growth efficiencies of 40–60% are generally considered realistic for the decomposition of soluble constituents; other compounds, such as waxes and cellulose, result in lower efficiencies. Aromatics such as lignin appear to be largely cometabolized by fungi. This involves enzymatic degradation of the substrate but little uptake of the breakdown products. The fungi gain little, if any, energy for growth and incorporate little C during the decomposition of the aromatics. Therefore, aromatic decomposition occurs only in the presence of available substrate. Where data are available only over extended periods, it is not possible to calculate true decomposition values and microbial growth efficiency because $CO_2$ is evolved from both the original substrate and the turnover of microbial cells.

## MULTICOMPARTMENTAL MODELS

The distinction between simple kinetic models and multicompartmental models is somewhat arbitrary since the sum of exponentials model above described C

mineralization from two pools or compartments. Generally, compartmental models are needed when a single equation is insufficient to describe the multiple transformation processes that occur simultaneously in soils. A multicompartmental model is depicted graphically as a set of boxes, each of which represents a pool or compartment. Most often, the pools are defined conceptually, but they can also be measurable fractions of SOM (see Alternative SOM Models). A series of arrows connecting the various pools represents transformations of organic matter or a nutrient element from one form to the other. The graphical representation of the model can be written as a series of simultaneous reactions. Some simple compartmental models can be solved analytically (e.g., in equation form), such as the sum of exponentials describe above, but as the models become more complex and include more compartments, it becomes necessary to solve them numerically.

The advent of computers permitted the solution of complex systems models that require iterative solving of multiple equations to address multicompartment dynamics. The modeling of soil biological processes began with ecologists working in natural ecosystems in the 1960s and 1970s. Early modeling in agricultural systems focused on crop production in response to physical parameters, rather than biological processes. A new emphasis in the 1970s on the environmental impacts of agriculture led to early models of N dynamics, including nitrate leaching and denitrification. Further emphasis on agroecology and SOM management in the 1980s and 1990s has led to the development of a large number of models of soil organic matter dynamics. Several reviews comparing many of these models are available (e.g., McGill, 1996; Molina and Smith, 1998; Paustian, 1994; Smith *et al.*, 1997). A subset of these comparisons is provided in Table 16.3.

Paustian (1994) classified multicompartmental models of SOM dynamics as either "process-oriented" or "organism-oriented." There are far fewer organism-oriented models (Table 16.3), which are sometimes called "food web models" and describe the flow of organic matter and nutrients through different functional or taxonomic groups of soil organisms. Process-oriented models are those that focus on the processes mediating the transformations of organic matter and nutrients, rather than on the activity of specific organisms or groups of organisms. In process-oriented model types, soil organisms, if present, tend to be represented as a generic biomass or as part of a pool of active SOM. This approach precludes the possibility of modeling changes in organic matter dynamics or nutrient cycling that might occur due to changes in the activity or composition of the soil organism community. Schimel (2001), however, points out that the microbiological underpinnings in process-oriented models are not absent, but are implicit and buried in the equation structure of the model as kinetic constants and response functions.

If most biochemical reactions in soils are mediated by enzymes and follow Michaelis–Menton kinetics, and if soil microbial populations grow and die back regularly, why is it that most process-oriented models use first-order kinetics to describe SOM and nutrient dynamics (Table 16.3)? As demonstrated previously, Monod or Michaelis–Menton kinetics can be simplified to first-order kinetics when substrate concentrations are sufficiently low. Several studies have shown that soil respiration usually occurs at 20 to 65% of its maximum potential rate.

**TABLE 16.3** Comparison of Basic Attributes of Several Multicompartmental Models of Soil Organic Matter and Nutrient Dynamics[a]

| Model | Resolution | | Litter/SOM distinction | Explicit decomposer pool | Nonliving SOM pools | | Regulation by soil texture | Rate kinetics | Other nutrient elements |
|---|---|---|---|---|---|---|---|---|---|
| | Spatial[b] | Temporal[c] | | | No. | Names | | | |
| CANDY (Franko *et al.*, 1995, 1997) | P, F, C | D, Y | Separate | No | 4 | Fresh organic matter, active SOM, stabilized SOM, inert SOM | Yes | First order | N |
| CENTURY (Parton *et al.*, 1987; Parton, 1996; Kelly *et al.*, 1997) | P, F, R, N, G | M | Separate | No | 5 | Metabolic litter, structural litter, active SOM, slow SOM, passive SOM | Yes | First order | N, P, S |
| DAISY (Hansen *et al.*, 1991; Mueller *et al.*, 1996) | P, F, C | H | Separate | No | 7 | Added OM 1, 2 (slow, fast); root OM 1, 2; SOM 1, 2; inert SOM | Yes | Michaelis–Menton and first order | N |
| DNDC (Li *et al.*, 1992a,b, 1997) | P | H, D | Separate | No | 5 | Very labile litter, labile litter, recalcitrant litter, humads—labile, humads—resistant | Yes | First order | N |
| ECOSYS (Grant *et al.*, 1993a,b; Grant 2001) | S, P, F | Mi, H | Separate | Yes | 7 | Soluble SOM, adsorbed SOM, microbial SOM, microbial residues, active SOM, passive SOM, particulate SOM | Yes | Monod | N, P |

(*Continued*)

TABLE 16.3—Continued

| Model | Resolution | | Litter/SOM distinction | Explicit decomposer pool | Nonliving SOM pools | | Regulation by soil texture | Rate kinetics | Other nutrient elements |
|---|---|---|---|---|---|---|---|---|---|
| | Spatial[b] | Temporal[c] | | | No. | Names | | | |
| Soil food web model (Hunt *et al*., 1984, 1987) | P, F | Mi, H | Separate | Yes | 4 | Labile litter, resistant litter, stable SOM, refractile SOM | No | Michaelis–Menton, logistic, and first order | N |
| ITE (Thornley and Verberne, 1989; Thornley and Cannell, 1992) | P, F | Mi | Separate | Yes | 2 | Biomass, SOM | No | Michaelis–Menton and first order | N |
| NCSOIL (Molina *et al*., 1983, 1997) | S | D | Separate | No | 5 | Litter Pool I—labile, Pool I—resistant, Pool II—labile, Pool II—resistant | No | First order | N |
| PHOENIX (McGill *et al*., 1981) | P, F | D, W, Mo | Separate | Yes | 4 | Metabolic litter, structural litter, humads, resistant SOM | No | Monod and pseudo-first order | N |
| Q-SOIL (Bosatta and Ågren 1985, 1994) | P, F | Y | Combined | No | 1 | Single pool | No | Pseudo-first order | — |
| RothC (Jenkinson *et al*., 1987; Jenkinson, 1990) | P, F, C, R, N, G | Mo | Separate | No | 4 | Decomposable litter, resistant litter, humus, inert SOM | Yes | First order | — |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| SOMM (Chertov, 1990; Chertov *et al*., 1997) | P, F, G | D | Combined | No | 3 | L—litter, F—humus/ organic debris, H—clay-bonded humus | No | First order | N |
| VVV (Van Veen and Paul, 1981; Van Veen *et al*., 1984) | P, F | D | Separate | No | 6 | Non ligniferous litter, ligniferous litter, protected active SOM, nonprotected active SOM, protected recalcitrant SOM, nonprotected recalcitrant SOM | Yes | First order | N |
| VVV (Verberne *et al*., 1990) | P, F | D | Separate | No | 6 | Labile litter, structural litter, recalcitrant litter, protected active SOM, nonprotected active SOM, old SOM | Yes | First order | N |

[a] With permission from Paustian (1994), McGill (1996), and Molina and Smith (1998).
[b] S, microsite; P, plot; F, field; C, catchment; R, regional; N, national; G, global.
[c] Mi, minutes; H, hours; D, days; W, weeks; Mo, months; Y, years.

This is generally low enough that using first-order kinetics would not introduce significant errors. The size of the microbial population remains implicit in the first-order kinetic constant $k'$ (as $X_0$ in the $\mu_{max}X_0/K_s$ term). A fundamental assumption of process-oriented models is that microbial processes are never limited by the size of the microbial population. In other words, there is always sufficient microbial diversity, and populations grow rapidly enough, to adapt to environmental stresses and changes in substrate supply. These assumptions are generally valid for ecosystem-level models of organic matter dynamics and decomposition; however, caution should be used when modeling specific processes performed by specific organisms, such as nitrification.

Most models of soil organic matter dynamics begin by modeling the decay of litter at the soil surface. The models assume that plant material contains a readily decomposable fraction (typically water-extractable material—sugars, soluble phenols, and amino acids) and a more resistant fraction containing cellulose and lignin (see Chap. 12 for additional information on C inputs to soil). Experiments have shown that the initial plant litter N, polyphenol, carbohydrate, and lignin contents control litter decay rates along with the abiotic temperature and moisture environment (higher decay rates with higher soil temperature and moisture conditions). Decomposition of different plant material for long periods (2 to 5 years) under various climatic conditions shows that 5–25% of the original C remains. The remaining material consists of plant lignins, microbial products, and humic material. The lignin:N ratio of plant material is positively correlated to the fraction of plant material resistant to decomposition. Most litter decay models include microbes that decompose plant material and form microbial products with a slow decay rate. Many of the models also assume that the majority of plant lignin (>70%) is directly transformed into organic material with a decay rate similar to that of microbial products. Native SOM located below ground is generally described using multiple pools to acknowledge its chemical heterogeneity. The number of pools included in each model varies significantly (Table 16.3). The division of SOM into various pools is based on stabilization mechanisms, bioavailability, and biochemical and kinetic parameters. Generally, the pools consist of at least one small "active" pool with a rapid turnover rate and one or more larger pools with slower turnover rates ranging from a few decades to thousands of years. The active pools are more closely associated with measurable entities such as microbial biomass, while the longer lived pools are generally more conceptual.

A detailed description of each model is beyond the scope of this chapter; however, we present the Rothamsted, Van Veen and Paul, and Century models as examples, with the focus on how they are constructed and on differences among them.

### Rothamsted Model

The Rothamsted model (ROTH) was developed using the long-term wheat plots in Rothamsted, England, and has more recently been used to simulate SOM dynamics at regional and global scales (Jenkinson and Rayner, 1977; Jenkinson *et al.*, 1992). The model represents five SOM fractions: (1) resistant plant material

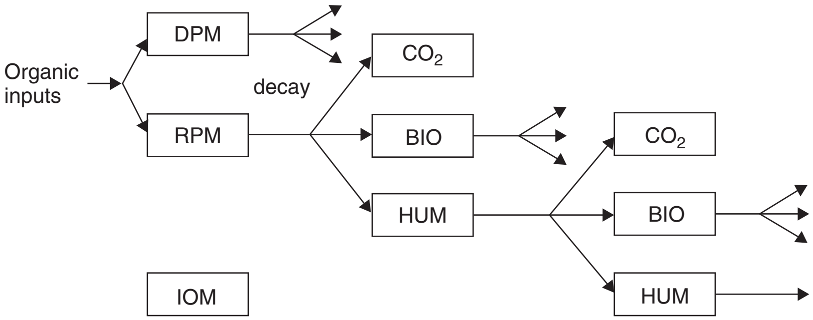



FIGURE 16.4 The ROTHC model showing the flow of carbon through the turnover model (with permission from Jenkinson, 1990). DPM, decomposable plant material; RPM, resistant plant material; IOM, inert organic matter; BIO, microbial biomass; HUM, humified organic matter.

($k$ = 0.03 year$^{-1}$), (2) decomposable plant material ($k$ = 4 year$^{-1}$), (3) microbial biomass ($k$ = 0.41 year$^{-1}$), (4) chemically stabilized SOM ($k$ = 0.0003 year$^{-1}$), and (5) physically stabilized SOM ($k$ = 0.014). The large number of data sets used to parameterize the model included incubations with $^{14}$C-labeled plant material (1- to 10-year periods), 1950–1990 thermonuclear radiocarbon generated by atmospheric bomb testing, microbial biomass estimates generated from chloroform fumigations, radiocarbon dating of SOM fractions, and measured long-term soil C levels at the Rothamsted plots. The decomposition rate of each of these components was assumed to be first order. It was also assumed that during decomposition each of the five components decayed to $CO_2$, microbial biomass, and humified organic matter in similar proportions (Fig. 16.4). The fit between the model and the experimentally determined results suggested that the model was a useful representation of the turnover of SOM in cropped soils. The model has since been tested using a variety of SOM data sets from cropping systems, including data sets from Rothamsted, England; western Canadian wheat–fallow systems; and sugarcane soils in Brazil.

The initial version of the Rothamsted model used measured C inputs to drive the model. However, later versions simulate plant production and nutrient cycling. More recently, Jenkinson (1990) added the impact of cation-exchange capacity on SOM stabilization. This alters the partitioning between $CO_2$ and biomass plus humus. Cation exchange capacity was preferred over clay content because it also accounts for changes in clay mineralogy. The new version of the model also replaced the small highly resistant pool ($k$ = 0.00035 year$^{-1}$) with an inert pool to account for the very old measured radiocarbon ages of SOM. Results from both the ROTH and the Century models suggest there is a considerable amount of uncertainty about the processes that control the formation of passive or inert SOM. Recent experimental results from Australia would suggest that charcoal from the burning of grass and trees is an important source of inert SOM. However, the necessity for an inert pool versus a very recalcitrant pool is still under debate.

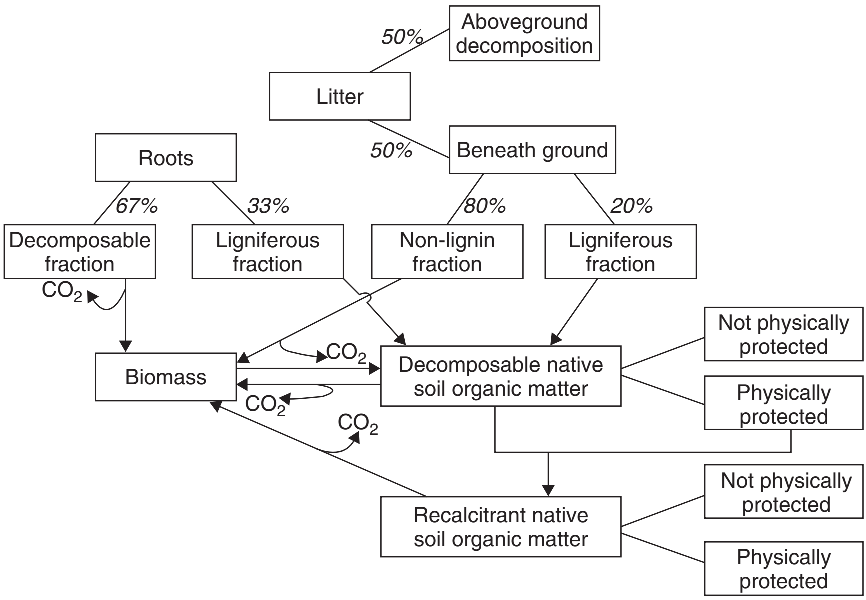



FIGURE 16.5 The structure of the Van Veen and Paul model for soil organic matter dynamics (with permission from Van Veen and Paul, 1981).

## Van Veen and Paul Model

Van Veen and Paul (1981; Van Veen *et al.*, 1984) developed a SOM model (Fig. 16.5) in which plant litter is divided into three fractions: (1) easily decomposable sugars and amino acids, (2) slowly decomposable cellulose and hemicellulose, and (3) resistant lignin. The soil C pools include: (1) microbial biomass, (2) decomposable SOM, and (3) recalcitrant SOM. The model assumes that microbes decompose the SOM and litter pools and that a certain fraction of the material decomposed will be stabilized into microbial biomass. Once dead, the microbial biomass can be considered as an easily decomposable pool (cytoplasm) and a more resistant component (cell wall). Table 16.4 shows the decomposition rate constant of the microbial biomass and the size of the microbial product pools. The size of the microbial product pools is small, as these values describe only the products formed from the recently added residue, as would be found in a $^{14}$C-labeling experiment. Having the model separately follow tracers such as $^{14}$C and $^{15}$N can yield a great deal of information about recently added components and results in rigorous testing of the model.

The model includes the impact of soil texture on soil C stabilization (higher stabilization with higher silt and clay content), direct transfer of lignin-like plant material to the decomposable SOM pool, and the concept that both decomposable and recalcitrant SOM is divided into physically protected and nonprotected fractions. The physically protected SOM fraction has a lower decomposition rate as a result of soil physical aggregation (harder for microbes to decompose plant material in

TABLE 16.4 Pool Sizes, Decomposition Rates, and Efficiency of Microbial Production Used in the Van Veen and Paul Carbon Turnover Model Describing the Decomposition of 1000 μg g$^{-1}$ of Soil[a]

| Pool | Residue carbon (μg g$^{-1}$ of soil) | Decomposition rate, *k* (day$^{-1}$) | Utilization efficiency (%) |
|---|---|---|---|
| Sugars, amino acids | 150 | 0.2 | 60 |
| Cellulose, hemicellulose | 650 | 0.08 | 40 |
| Lignin | 200 | 0.01 | 10 |
| Decomposable microbial products[b] | 6 | 0.8 | 40 |
| Recalcitrant microbial products[b] | 4 | 0.3 | 25 |
| Active protected soil organic matter | 5000 | $3 \times 10^{-4}$ | 20 |
| Old soil organic matter | 7000 | $8 \times 10^{-7}$ | 20 |

[a] With permission from Voroney *et al*. (1981).

[b] Pool sizes shown refer to those produced from the added substrate alone. Actual total values in soil are much larger.

the middle of the soil aggregate). The model also assumes that cultivation of the soil will increase the fraction of decomposable and recalcitrant pools in the non-physically protected fraction (soil aggregate disruption). Clay type, such as allophane, and the soil silt and clay content control stabilization of SOM. The size of the microbial biomass is largely controlled by the total soil C content, recent substrate additions, and clay content.

### The Century Model

The Century model (Fig. 16.6), originally developed to model SOM dynamics in the High Plains of the United States (Parton *et al*., 1987, 1994), divides fresh organic residues on the basis of the lignin to N ratio. Structural components comprising lignin, cellulose, and hemicellulose have a decomposition rate of 0.011 day$^{-1}$, metabolic components have a *k* of 0.03 day$^{-1}$. Lignin content is also used to control the decay rates of the structural components where high lignin results in slower decay rates because lignin is closely associated with the cellulose and hemicellulose components and has a protective effect on them. Surface litter is shown to enter a surface microbial pool. Buried litter and roots feed into an active C pool, which comprises microbial biomass and microbial metabolites. The slow C pool ($k = 0.00054$ day$^{-1}$) has an intermediate turnover rate and receives some C directly from the lignin plant components. The arrows showing the $CO_2$ evolved during each transformation are indicative of the microbial growth efficiencies discussed earlier. The first-order decay rates for each of the pools, given in Fig. 16.6, correspond to turnover times of roughly 3 and 0.5 years for the structural and metabolic components, 1.5 years for the active fraction, 25 years for the slow, and 1000 years for the passive pools. The Century model can utilize either monthly or daily precipitation and air temperature data and soil-specific values such as sand, silt, and clay contents; bulk density; soil depth; and total soil C and N contents.

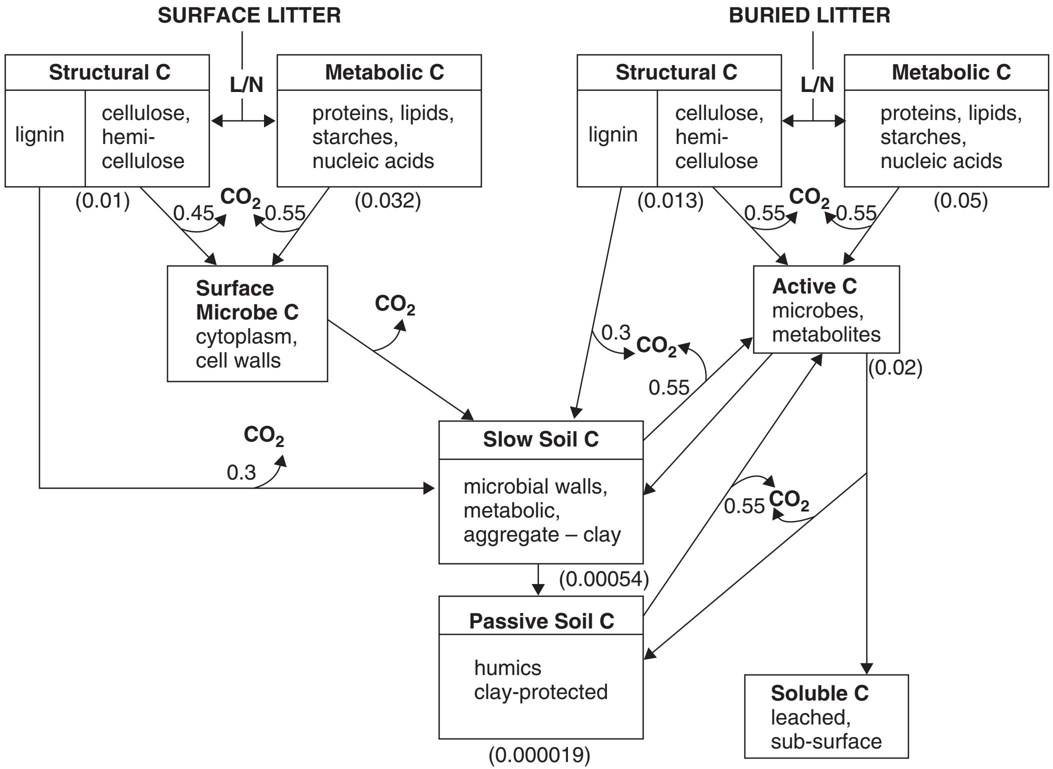



FIGURE 16.6 The Century model showing lignin and N controls on the proportion of plant residue structural and metabolic components. Carbon flows entering microbial biomass and three soil components are controlled by decomposition rates ($day^{-1}$) shown in brackets. Microbial utilization efficiencies are shown on $CO_2$ loss arrows (with permission from Parton *et al.*, 1994).

The silt and clay contents play major roles in protecting the slow C pool, while clay content controls formation of passive soil C.

## ALTERNATIVE SOM MODELS

With a few exceptions, the pools used in the SOM dynamics models described above are functional and kinetically defined. While the microbial biomass and the litter inputs are relatively easy to measure, it is difficult to define a "slow" pool of organic matter in chemical or physical terms. While the multicompartmental models generally work well at predicting measured changes in total SOM, it is difficult to initialize models using independent measures and therefore they rely on site-specific calibrations based on total organic matter levels. Recent attempts have been made to link conceptual pools of SOM with measurable fractions, leading Elliott *et al.* (1996) to use the phrase "modeling the measurable or measuring the modelable." Such attempts include developing chemical or physical fractionation procedures to match measurable fractions to model pools (e.g., Sohi *et al.*, 2001) or to construct new models that coincide with measurable fractions. Many of the model structures being proposed attempt to account for the various protection mechanisms that stabilize incoming organic matter. One such example is illustrated in Fig. 16.7. However, the development of models based on physical fractions is still in its infancy and has yet to overcome some of its challenges. Smith *et al.* (2002) have suggested that a measurable fraction and model pool are equivalent only when the measured fraction is unique and noncomposite. To date, few models that have been developed and tested would meet these criteria.

It is recognized that SOM is a mixture of a very large number of organic compounds with widely differing chemical and physical properties. The models described above partition SOM into only a few discrete pools. Each pool is treated as having constant characteristics, thus the changing dynamics of the whole organic matter follows from the changing distribution among the various pools. This leads to straightforward models, but their theoretical underpinning can be questioned. An alternative approach is to describe SOM as a continuum from fresh plant residues to refractory humic substances. Bosatta and Ågren proposed that the heterogeneity of SOM could be described using a quality variable ($q$), which determines the decomposition rate (Ågren and Bosatta, 1998; Bosatta and Ågren, 1985, 1995). In their model, $q$ for each cohort of new organic matter varies over time according to a continuous-quality equation. The equation describes changes in the molecular accessibility of a C atom to decomposers as the microbial population assimilates organic compounds and utilizes them to produce compounds of different quality. While the QSOIL model is conceptually satisfying, its complex mathematics has limited its broad application. Yang and Janssen (2000) propose using a single-pool, first-order model that allows the rate constant ($k$) to change. This approach is attractive because it is derived only from measured quantities of remaining organic material and there is no need for other variables that cannot be measured. The key feature of the model is that it takes into account the decrease

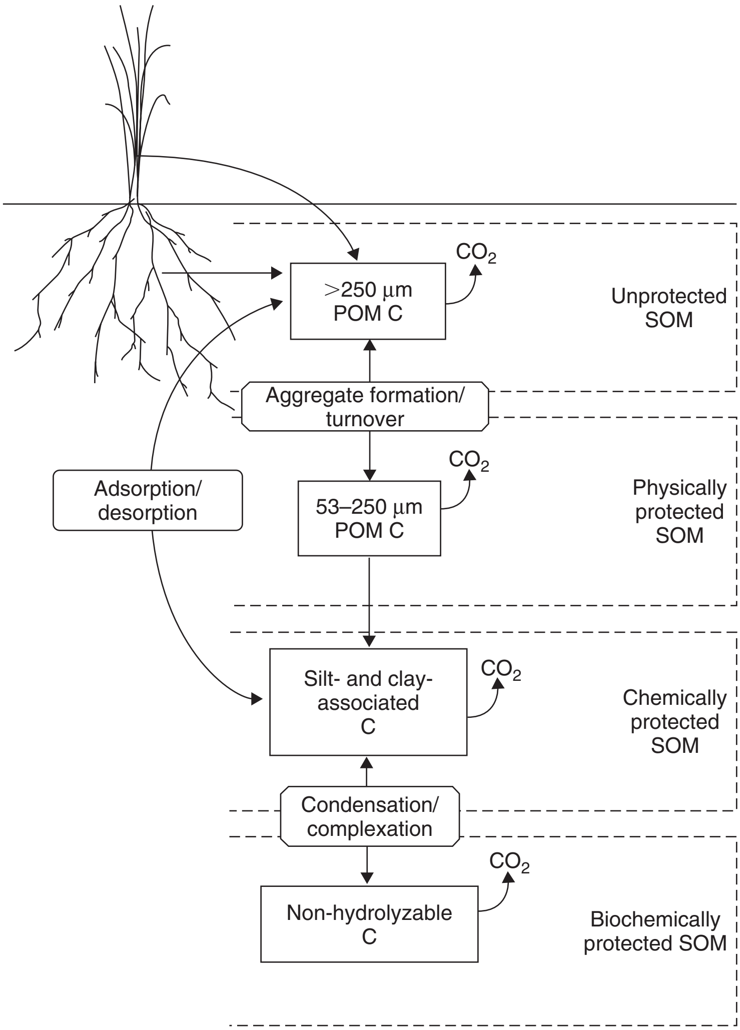



FIGURE 16.7 A soil organic matter dynamics model based on measurable fractions that account for various protection mechanisms (with permission from Six *et al*., 2002).

over time of the average and actual mineralization rates, which the authors refer to as "aging."

## MODELS OF NON-C NUTRIENT ELEMENTS

Nutrient cycles in soil are tightly coupled through the nutrient demands of the microbial biomass during decomposition. For this reason, several of the C-centric multicompartmental models of SOM dynamics are able to model nutrient elements such as N, P, and S (Table 16.3). Models that describe the dynamics of

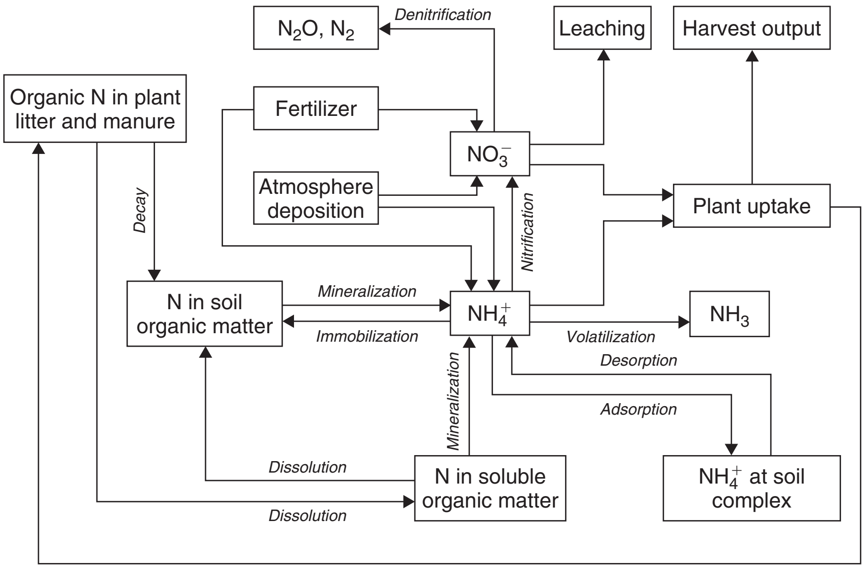



FIGURE 16.8 The structure of the ANIMO N submodel for soil nitrogen dynamics (with permission from Wu and McGechan, 1998).

nutrient elements differ in scale and scope from the models of SOM dynamics. While SOM models evolved out of ecological and environmental perspectives, models of nutrient dynamics evolved primarily from the desire to improve agronomic crop production and fertilizer use efficiency. More recently, nutrient models have been extended to the study of environmental pollution from excess fertilizer or the land spreading of manures and wastes.

Frissel and Van Veen (1982) classified models of soil N in terms of (1) their purpose, prediction, management, or scientific understanding; (2) their time span; (3) whether they were budget-based or dynamic models; and (4) whether the models were dominated by transport processes, SOM dynamics, or soil–plant relations. Models of soil N dynamics generally include descriptions of physical processes such as the transport of water, solutes, heat, and gases; biological processes such as mineralization, immobilization, nitrification, and denitrification; and physicochemical processes such as volatilization, adsorption, and fixation. Model structures of soil N models, for example ANIMO illustrated in Fig. 16.8, generally resemble the conceptual depiction of the soil N cycle. A large set of models of N turnover in the soil–plant system, both stand-alone models and submodels, have been compared by running simulations on the same data set (de Willigen, 1991), similar to the comparison of SOM models performed by Smith *et al.* (1997). The conclusions of the N model comparison were that the models adequately predicted aboveground processes such as plant uptake of N and dry matter production, but the simulation of belowground microbial N transformations was the most problematic. Wu and

McGechan (1998) provide a more detailed examination of four N dynamics models (SOILN, ANIMO, DAISY, and SUNDIAL), in which they focused on the equations representing the constituent processes. SOILN was found to have the most detailed treatment of plant uptake. ANIMO had the most complex treatment of animal slurry and also the most mechanistic representation of denitrification. In both comparisons, the models demonstrated individual strengths and weaknesses, but in general they are more similar than they are different.

Phosphorus cycling models have been developed and incorporated into plant–soil ecosystem models. Similar to N, the demand for P cycling models arose because soil P availability is the major nutrient limiting plant production in many tropical systems and, conversely, because P from nonpoint sources such as agricultural soils has a major environmental impact on water quality. Despite this, few models calculating long-term changes in soil P have been developed partly because of the many complicated solid-phase interactions of P sorption to minerals over and above the biological transformations. Jones *et al.* (1984) originally developed routines for simulating soil P dynamics, which became incorporated into the EPIC (erosion–productivity impact calculator) model. These routines have since been incorporated into several other models that describe the transport of soluble and particulate P, adsorption and desorption, mineralization and immobilization between organic and inorganic forms, leaching, plant uptake, and runoff. As an example, the model structure of the GLEAMS P model is illustrated in Fig. 16.9. Lewis and McGechan (2002) compared four of the published P cycling models (ANIMO, GLEAMS, DAYCENT, and MACRO) and concluded that all the existing models have substantial limitations and that a hybrid submodel combining the best features of these models needs to be developed. Unfortunately, there are limited data

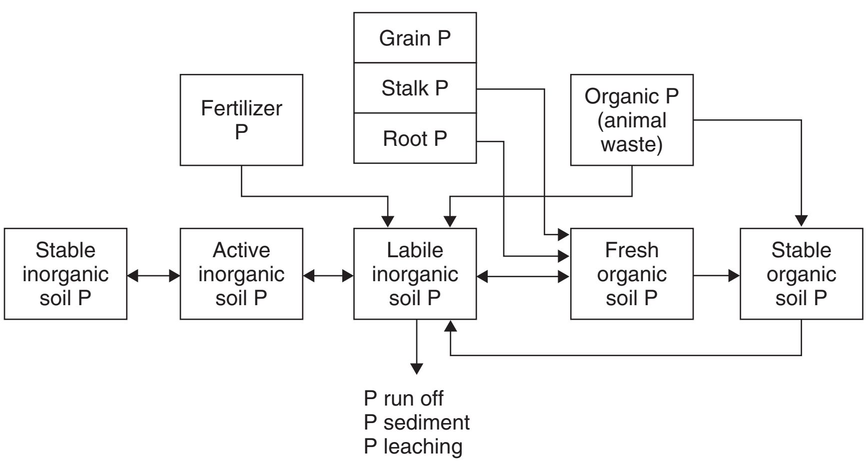



FIGURE 16.9 The structure of the GLEAMS P submodel for soil phosphorous dynamics (with permission from Lewis and McGechan, 2002).

available to describe the critical process represented in P cycling models, thus limiting the accuracy of existing and new P cycling models.

## ECOSYSTEM MODELS: INTERACTIONS OF NUTRIENT CYCLING AND SOM DYNAMICS

The initial computer models of SOM dynamics were expanded in the 1980s to include nutrient cycling and plant production submodels. The most recently developed SOM models are capable of simulating losses of nutrients from leaching of $NO_3^-$ and dissolved organic matter and nitrogen gas losses ($N_2O$, $N_2$, and $NO_x$) from nitrification and denitrification. These models have also been incorporated into long-term models of soil development (500,000 to 4 million years), which include plant production, soil N and P dynamics, and SOM dynamics. Applied to a scenario in the humid tropics, the century model correctly demonstrated that N is the primary limiting factor for plant growth during early soil development (20,000 to 300,000 years), while P limits plant growth for old soils (>550,000 years).

Ecosystem-level models are generally a hierarchical construction of model subcomponents (Fig. 16.10). Each of the model subcomponents can consist of a model representing more detailed processes. The figure demonstrates that microbial transformations have a big impact on available soil nutrients and that available soil nutrients then impact nutrient uptake by the plants, which modifies plant production and crop yields. Climate, land use, and soil physical information are drivers for plant production, soil water transport, and microbial transformations. The hierarchical construction provides a means of connecting various components into a single cohesive model that can examine the large number of interacting parameters required to examine processes at large spatial and temporal scales. However, the complexity of the agroecosystem models can make it difficult to verify that the results are reasonable. For example, the failure of the model to properly simulate denitrification $N_2O$ and $N_2$ gas fluxes can result from an error in estimating plant demand for $NO_3$ and $NH_4$, and net uptake of $NO_3$ and $NH_4$ by soil microbes, or errors in simulating soil water content. Generally, models that are applied at larger spatial and temporal scales use input data of lower resolution; therefore errors in estimating the validity of model output are not easily scaled up and become difficult to estimate.

Both data and modeling studies show that there are strong interactions between nutrient availability and SOM dynamics. The Century model can simulate the interactions among plant production, nutrient cycling, and SOM dynamics. Paustian *et al.* (1992) used field-plot data from a 40-year agricultural experiment in Sweden (see Table 16.5) to test the Century model and demonstrate the interactive impact of adding different types of organic matter (sawdust, green manure, farmyard manure, and straw) and inorganic fertilizer on plant production and nutrient uptake, nutrient cycling, and SOM dynamics. The results for the low N content (<0.5% N) organic additions (straw and sawdust) show that there is a decrease in plant N uptake for sawdust and no change for straw, while soil N and C levels are higher compared to the control treatment (no addition). The decreased

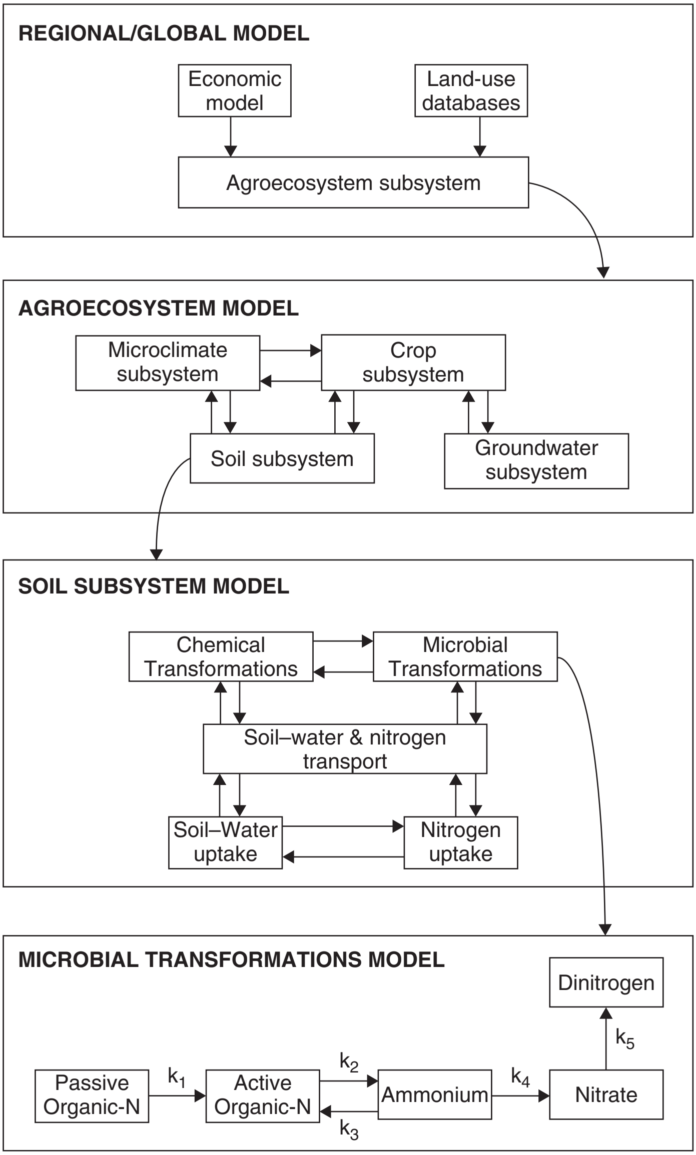



FIGURE 16.10 An example of the hierarchy of submodels used in the construction of complex predictive models for regional and global scales (with permission from Paustian, 2001; Rao *et al.*, 1982).

plant production with sawdust addition is a result of immobilization of N. Adding inorganic N fertilizer causes plant production and N uptake to be increased by >90%, while soil C and N are increased relative to the no-addition control (lower increases compared to the straw and sawdust treatment). The increased soil

**TABLE 16.5** Observed versus Simulated Plant N Uptake and Change in Soil N and C (1990–1956) for Different Organic Matter Management Practices for a Site in Sweden[a]

| | Fallow | No amendment | Fertilizer N | Straw | Green manure | Farm manure | Sawdust |
|---|---|---|---|---|---|---|---|
| Plant N uptake ($g N m^{-2}$) | | 4.7 (4.1) | 9.4 (9.3) | 4.7 (4.0) | 8.7 (8.6) | 6.9 (7.3) | 3.6 (2.1) |
| Change in soil N ($g N m^{-2}$) | −100 (−97) | −56 (−85) | −25 (−35) | +6 (−12) | +38 (+52) | +119 (+48) | +25 (+46) |
| Change in soil C ($g C m^{-2}$) | −700 (−720) | −800 (−790) | −200 (−250) | 0.0 (+300) | +300 (+500) | +1000 (+950) | +500 (+560) |

[a] Simulated values were generated by the Century model and are presented in parentheses. With permission from Paustian *et al*. (1992).

C and N levels result from increases in root C inputs. The high-N-content organic additions (farmyard manure and green manure) result in large increases in plant production and N uptake and substantial increases in soil C and N levels. Approximately 40% of the added N from the inorganic fertilizer and high-N organic additions goes into increased plant N uptake, while organic N additions result in greater stabilization of N in the soil (40% vs 20% for inorganic N fertilizer). Comparison of model results with observed data is quite favorable; however, more information will be needed to accurately predict the role of SOM in controlling global $CO_2$ levels.

## ESTABLISHING POOL SIZES AND KINETIC CONSTANTS

The various forms of organic matter in the soil–plant system and the complex changes that it undergoes can be rationalized using a pool structure. A pool can be defined as a compartment containing material of unique and uniform composition. An example of biological and chemical pool sizes can be found in the analysis of two Swedish agricultural systems (Table 16.6). The standing crop of barley fertilized with 120 kg N $ha^{-1}$ is greater than that of alfalfa, but the primary production of alfalfa is greater when one considers both the two harvests per annum and the root turnover in the alfalfa crop. The shoot-to-root ratio of 6:1 for barley is typical of many agricultural crops. The perennial alfalfa with more roots than shoots is representative of perennial crops. The microbial biomass is equivalent to about 50% of the weight of the plant standing crop and greatly exceeds the metazoan biomass. The uncultivated alfalfa has a soil fauna biomass of 105 kg C $ha^{-1}$ compared to the 55 kg C $ha^{-1}$ in the barley field. The soil organisms account for slightly more than 3% of the total soil C in both systems, and direct microscopy of the bacteria and fungi shows the predominance of fungal biomass. The annual C balance of the two systems (Table 16.7) shows the root respiration as 11–12%

**TABLE 16.6** Pool Sizes of Biotic Components in or above the Top 27 cm of Soil in Two Swedish Cropping Systems (kg C $ha^{-1}$)[a]

| | Cropping system | |
|---|---|---|
| Component | Barley | Alfalfa |
| Plant shoots and surface litter | 4100 | 3200 |
| Aboveground metazoan fauna | 0.8 | 1.6 |
| Roots | 700 | 4000 |
| Fungi | 2300 | 1800 |
| Bacteria | 900 | 900 |
| Belowground metazoan fauna | | |
| Arthropods | 1.5 | 6.2 |
| Nematodes | 1.3 | 2.4 |
| Enchytraeids | 3.4 | 2.7 |
| Earthworms | 14 | 44 |
| Total fauna | 55 | 102 |
| Soil organic C | 102,000 | 86,000 |
| Soil organic N | 10,200 | 9600 |

[a] With permission from Paustian *et al*. (1990).

**TABLE 16.7** The Annual Carbon Balance Sheet of Two Swedish Cropping Systems[a]

| | Barley | Alfalfa |
|---|---|---|
| Carbon input | | |
| Primary production | 5400 | 9000 |
| Carbon output | | |
| Harvest | 3000 | 3500 |
| Root respiration | 600 | 1100 |
| Faunal respiration | 170 | 310 |
| Microbial respiration | 1510 | 2620 |
| Total outputs | 5280 | 7530 |
| Net annual change | +120 | +1470 |

[a] With permission from Paustian *et al.* (1990).

of the net primary production. Microbial respiration dominates faunal respiration by a ratio of 9:1, with heterotrophic $CO_2$ evolution returning 32% of the primary production to the atmosphere. The annual change in the residues in the alfalfa field is attributable to the buildup of the plant residues in the perennial system.

Studies of substrate degradation in soil require both short intervals between early measurements and extension of the measurements over time intervals long enough to capture the full range of turnover times. Several tools are available for determining the pool sizes and dynamics of SOM fractions. The soluble components, and even some of the cellulose of plant residues, decompose within hours to days. If measurements are delayed, the degradation of the microbial products,

rather than that of the original substrate, is measured. Incubations in the laboratory or in the field, extending to hundreds of days or longer, are needed to evaluate the more persistent components. Generally, incubations incorporating respiration measurements and curve-fitting analysis are sufficient for determining the short-term dynamics. Soil organic matter components also occur in intermediate pools with turnover times of 10–100 years. In some cases, the turnover rates of these constituents can be measured by using the stable isotope $^{13}C$ (Balesdent *et al.*, 1987). This isotope accounts for 1.1% of the $CO_2$-C of the air. Plants with the $C_3$ photosynthetic pathway discriminate more against this isotope than do plants with the $C_4$ pathway, resulting in differential $^{13}C$ enrichment of plant materials. Residue inputs and SOM turnover can be measured using mass spectrometry in soils formed under $C_3$ plants (cool season grasses, trees) if $C_4$ plants such as maize or sorghum are then grown or if residues from these plants are added. The reverse sequence of crops will also make measurements possible. The natural abundance of $^{13}C$ in the plants and soil may provide insufficient differentiation. In this case, artificially labeling plant materials with $^{13}C$ by growing them in closed chambers with enriched $^{13}CO_2$ or $^{14}CO_2$ may provide a better tracer. Studies examining the transformations of N, such as plant uptake or mineralization, can be done using the $^{15}N$ stable isotope tracer.

The slow decomposition rates of the most resistant fractions, making up 50% of the soil C and persisting for hundreds to thousands of years, are not easily measured with normal tracer techniques. For this, we resort to C dating, which utilizes the much longer half-life of naturally occurring $^{14}C$ (Paul *et al.*, 1997). Such studies have found that the average age (mean residence time) of organic matter in the surface of temperate agricultural soils ranged between modern and 1100 years, with an average of 560 years. Deeper in the soil profile (50–100 cm), the average age was 2757 years and ranged from 1500 to 6600 years. It is important to note that studies of nutrient and organic matter turnover are best conducted on well-characterized, long-term plots where the yield or primary productivity components, soil type, and long-term climate and management controls are known.

## MODEL SELECTION AND EVALUATION

As mechanistic soil–crop–water–atmosphere models become increasingly accepted as tools for analyzing agronomic or environmental issues, users are being faced with an increasing number of models to choose from. Ideally, a simulation model would include all of the processes dictating the dynamics of SOM or nutrient elements at a level of detail that represents the current state of the art in understanding, and model selection would be a moot point. Unfortunately, an ideal model for complex and heterogeneous agroecosystems is impractical. Not all system processes are fully understood and thus each model includes various approximations and simplifications. In addition, the documentation available for each model generally does not refer to the validity, limits, and potential applications of the model, which would provide some guidance in the selection of the most appropriate model.

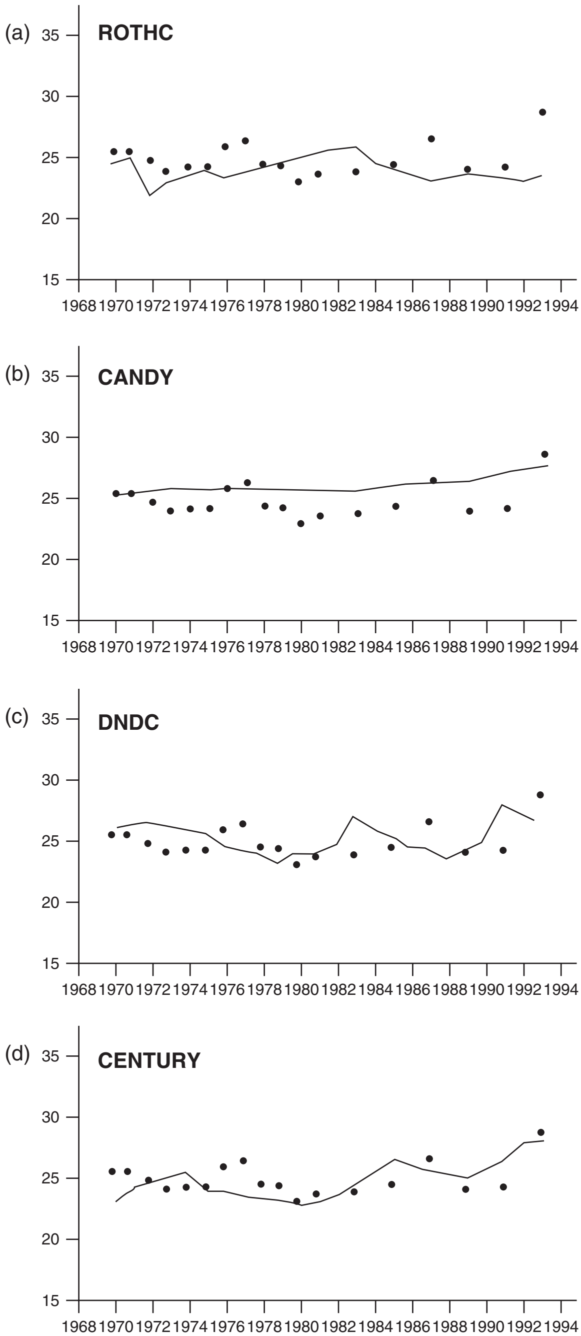



FIGURE 16.11 Observed vs simulated soil carbon levels for the Century, ROTHC, CANDY, and DNDC models at the Tamworth lucerne/clover crop rotations (with permission from Smith *et al.*, 1997).

So how does one select the "best" model to use? Smith *et al.* (1997) use many of the above statistical criteria to evaluate the effectiveness of different soil organic matter models to simulate soil organic matter dynamics at different sites around the world. This paper discusses the usefulness of these different statistical criteria and demonstrates that six of the nine models considered in the model comparison did an equally good job of representing the observed soil organic matter dynamics. Figure 16.11 shows the comparison of observed and simulated soil carbon levels for four of these models with the Tamworth lucerne/clover crop rotation system (Smith *et al.*, 1997).

Some criteria that may be used in the selection of an appropriate model include (Rao *et al.*, 1982): the intended use of the model, the spatial and temporal scales of application, the availability of input data, and the availability of computational facilities.

Once a model has been selected and applied, the output must be evaluated by comparing simulated or predicted values with actual measured or observed values from field experiments not used originally to develop the model. At its simplest, model evaluation can be done visually or graphically. This approach provides a rapid and easy means of evaluating whether a model is producing results close to those observed. One problem associated with the approach is that the difference between predicted and observed values cannot easily be quantified. Loague and Green (1991) suggest that model evaluation should include both qualitative graphical and quantitative statistical approaches. Several statistical criteria for evaluating simulated results are outlined in Table 16.8. Maximum error represents the

TABLE 16.8 Statistical Criteria for the Evaluation of Model Performance[a]

| Criterion | Formula[b] | Optimum |
|---|---|---|
| Maximum error | $Max\left\|P_i - O_i\right\|_{i=1}^{n}$ | 0 |
| Root mean square error | $\frac{100}{\bar{O}}\sqrt{\sum_{i=1}^{n}(P_i - O_i)^2/n}$ | 0 |
| Modeling efficiency | $\left(\sum_{i=1}^{n}(O_i - \bar{O})^2 - \sum_{i=1}^{n}(P_i - O_i)^2\right)\Big/\sum_{i=1}^{n}(O_i - \bar{O})^2$ | 1 |
| Coefficient of determination | $\sum_{i=1}^{n}(O_i - \bar{O})^2\Big/\sum_{i=1}^{n}(P_i - \bar{O})^2$ | 1 |
| Coefficient of residual mass | $\left(\sum_{i=1}^{n}O_i - \sum_{i=1}^{n}P_i\right)\Big/\sum_{i=1}^{n}O_i$ | 0 |

[a] With permission from Loague and Green (1991).
[b] $P_i$, predicted value $i$; $O_i$, observed value $i$; $\bar{O}$, mean of observed values; $n$, number of data pairs.

single largest difference between a pair of predicted and observed values. The root mean square error represents the total difference between the predicted and the observed values, proportioned against the mean observed value. Modeling efficiency (EF) assesses the accuracy of simulations by comparing the variance of predicted from observed values to the variance of observed values from the mean of the observations. The EF is essentially a comparison of the efficiency of the chosen model to the efficiency of a very simple predictive model: the mean of the observations. The coefficient of determination is a measure of the proportion of the total variance in the observed data that is explained by the predicted data. Last, the coefficient of residual mass gives an indication of the consistent errors in the distribution of all simulated values across all measurements.

Ultimately, a model that performs well (i.e., in which modeled output matches observations reasonably well) is not necessarily a "good" model. A good model is conceptually clear and can be easily communicated to others. A bad model may perform well, but because it can be inspected and modified only with great difficulty, there is no way to determine if the validation is pure chance or something meaningful. It is important that the user understand the assumptions and limitations of a selected model at each stage of its use and application.

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PART

# V

# Soil Organisms: Man and Nature

# 17

# Management of Organisms and Their Processes in Soils

Jeffrey L. Smith
Harold P. Collins



## INTRODUCTION

Historically humans have managed organisms inadvertently, consciously, and sometimes by simple intuition. One of man's first introductions to a microbial process occurred over 100,000 years ago when fruit fermented and formed wine (Purser, 1977). Reference to wine-making methods were recorded on ceramic artifacts dating to 5000 years BCE. Enhancements to wine making and viticulture were made during the times of the Egyptians, Romans, and Greeks, who also promoted its use in everyday life. It was not until the mid-1800s that Louis Pasteur discovered the process of fermentation, shedding light on the thousands of years of humans managing organisms to their benefit (Forester, 1998). A similar story could be told of cheese and cheese making, as archaeologists have evidence of cheese consumption dating back to 6000 years BCE and depictions of it on Egyptian tomb murals dating to 2000 BCE (Smith, 1995). Management of organisms for

other human foodstuffs includes yeast (*Saccharomyces cerevisiae*) in bread making and the cultivation of mushrooms.

Managing microorganisms can mean combating pathogenic or infectious organisms or promoting beneficial organisms or their products. Ancient Chinese and Arab cultures developed a procedure for scraping sores of people infected with mild cases of smallpox and infecting healthy people to ward off a more serious case of the disease. This practice was eventually introduced in Western Europe in the 1800s when Edward Jenner inoculated people with cowpox germs as a prevention against smallpox (Wilson, 1976). Jenner termed the procedure vaccination, from the Latin word *vacca*, for cow. However, the rational basis for vaccination came from Louis Pasteur's experiments with chicken cholera. Pasteur could have used the phrase he coined, "Chance only favors the prepared mind," to describe another manipulation of organisms that led to the discovery and production of penicillin. The accidental contamination of a culture plate of staphylococci by a mold (*Penicillium notatum*) led to the discovery and naming of penicillin by Sir Alexander Fleming in 1928 (Wilson, 1976; Forester, 1998). This discovery led to the production of penicillin as an antibacterial drug 12 years later by E. Chain and H. W. Florey. This touched off a worldwide search for soil organisms with antibiotic characteristics and the discovery of streptomycin by Dr. Selman Waksman, a soil microbiologist.

Since agriculture has been practiced for thousands of years, it is no surprise that examples of managing and manipulating microorganisms are abundant. Farming, especially in the uplands of Greece, intensified around 800 BCE, giving rise to erosion and decreased fertility (Encyclopedia Britannica, http://www. search. eb.com). As time went on, the Romans continued the practice of clearing forests and excessive grazing and cultivation of the land. However, the Romans recognized that to help fertility, the land needed to be fallowed at some time. They also were aware that to boost fertility, they needed to rotate crops and add lime and manure (Hillel, 1992). They found that growing alfalfa and clover added fertility, as did using green manures such as lupines, but they did not know why. Parallel with agriculture development was the practice of composting, in which a soil pit was maintained with human and animal waste, weeds, leaves, and household waste and watered regularly. The decomposed material was used as fertilizer and mulch that improved the physical, chemical, and biological characteristics of the soil. The management of microorganisms extends to plant pathology as well. For centuries it was known that there was an association between barberry and stem rust of grain (Walker, 1950). In the 1600s, farmers sought to adopt a barberry-eradication measure without knowing the cause-and-effect relationship. Likewise with simple observation and intuition, farmers began using salt brine to control wheat bunt.

These few examples depict a human population that has been managing microorganism and their processes for thousands of years. Many of the agricultural, forestry, and rangeland practices we use today actually have deleterious effects upon microorganisms and their processes. Practices including plowing, clear-cutting forests, and overgrazing of rangeland decrease organism populations and promote nutrient loss, with an overall result of decreasing soil quality. Humans as caretakers

of the land must reverse land degradation to increase soil quality and ecosystem health to provide food and fiber for a growing world population. We can begin this long journey by developing ways in which to manage organisms and their beneficial processes in soil systems.

## CHANGING SOIL ORGANISM POPULATIONS AND PROCESSES

Soil organic matter (SOM), a direct result of microbial activity, plays a role in terrestrial ecosystem development and functioning. In both undisturbed and cultivated systems, potential productivity is directly related to SOM concentrations and turnover. The dominant effect that SOM has on ecosystem structure and stability is clear evidence for the need to protect current organic matter levels and to develop management practices that will enhance soils with declining SOM contents (see Chap. 12 for more detail on organic matter). Organic matter contents in soils range from less than 0.2% in desert soils to over 80% in peat soils. In temperate regions, SOM ranges between 0.4 and 10.0%, with soils of humid regions averaging 3–4% and those in semiarid areas 1–3%. Although it is only a small fraction of the soil, components of SOM are the chief binding agents for soil aggregates that, in turn, control air and water relations for root growth and provide resistance to wind and water erosion. With 95% of soil nitrogen (N), 40% of soil phosphorus (P), and 90% of soil sulfur (S) being associated with the SOM fraction, decomposition and turnover can supply most macronutrients needed for plant growth (Smith and Elliott, 1990; Smith *et al.*, 1992). During decomposition, microorganisms assimilate complex organic substrates for energy and carbon (C) and release inorganic nutrients. This process is controlled by temperature, moisture, soil disturbance, and the quality of SOM as a microbial substrate. These factors, together with the size and activity of the microbial population, regulate the rate of decomposition and nutrient release (Smith and Paul, 1990; Smith, 1994).

The two most significant regional ecosystem disturbances that directly decrease SOM are tropical and boreal deforestation and the intensive cropping of the world's prime farmland and forests. Forests and agricultural lands contain 90% of terrestrial C and are responsible for 80% of the yearly primary C production (Smith and Paul, 1990). The decrease in SOM is paralleled by declines in soil productivity and contributes to increasing global $CO_2$ concentrations. In both forest and agricultural ecosystems, the management of plant residues is necessary to prevent SOM decline and possible ecosystem collapse.

Recent years have witnessed increased concern about environmental pollution from synthetic chemicals and disposal of wastes. Problems with toxic waste dumps, garbage landfills, and acid rainfall have focused attention on the soil, the most widely used depository. The soil as a depository is a perturbed system, experiencing gross changes in temperature, moisture, and biological activity. Controlling factors and processes may be drastically altered under these circumstances, causing

chemical cycling, ecosystem stability, and system resiliency to shift in unpredictable ways.

## TILLAGE AND EROSION

Tillage affects the amount of SOM buildup or loss in two ways: (1) through the physical disturbance and mixing of soil and the exposure of soil aggregates to disruptive forces and (2) through incorporation and distribution of plant residues in the soil profile. The degree of residue incorporation has a major effect on microbial activity and the rate of decomposition. Decomposition rates of residues are generally slower when left on the soil surface than when buried in soil. The combination of reduced litter decomposition rates and less soil disturbance usually results in greater amounts of SOM in reduced tillage vs conventionally tilled systems (Dalal *et al.*, 1991). Tillage also alters the physical and chemical properties of the soil environment by affecting water content and aeration, as well as the microclimate near the soil surface. These in turn regulate the soil biota and the biological processes they mediate. Conventional cropping practices with intensive tillage simplify microbial community structure, resulting in lower stability or resiliency in function. For example, reductions in stability have led to gaps in biochemical functions related to N transformations during periods of environmental stress or when plants are not present. Reduced tillage and cover crops generate soil-litter conditions very different from those of conventional systems. The distributions and types of residues modify microbial biomass, pathogen, and nematode and insect community structure and regulate microbial mineralization/immobilization rates controlling the loss of C and N from soil (Curci *et al*., 1997; Calderon *et al*., 2000).

Between 35 and 50% of the SOM and N were lost during the first 50 years of tillage in the Great Plains of the United States (Bauer and Black, 1981). This loss is most rapid during the first few years of cultivation, and eventually an apparent equilibrium is established, provided constant management practices are employed. The time required to reach this equilibrium will vary depending on climatic conditions, extent of erosion, type and rate of residue return, and soil type.

Over a 14-year period in Canada, cultivated, brown, prairie soils lost 26% of their SOM and 33% of their N. In the next 20 years, losses decreased by half (Doughty *et al*., 1954; Campbell *et al*., 1975). In other Canadian soils, over a 60- to 80-year period, C and N losses ranged from 50 to 60% and 40 to 60%, respectively (Campbell *et al*., 1976; Voroney *et al*., 1981). There is substantially less information concerning the effects of cultivation on chemical and physical soil properties in semiarid tropical regions. A 1986 study (Dalal and Mayer, 1986) found that soils in southern Queensland, Australia, had lost 36% of their C and N over a 20- to 70-year period and that numerous fertility parameters related to SOM levels were declining. Losses of C and N from tillage are usually determined by comparing cultivated with native uncultivated systems, but other factors are also involved in these losses. The effect of tillage in the long term is to decrease the SOM level, which, in turn, disrupts nutrient cycling and fertility and degrades soil quality.

TABLE 17.1 Ratios of Nutrients and Microbial Populations in the 0- to 7.5-cm Soil Depth of No-Tillage, Conventional Tillage, and Conservation Reserve Program Soils

| Soil parameter | NT:CT[a] | CRP:CT[b] |
|---|---|---|
| Total carbon | 1.25 | 0.99 |
| Total nitrogen | 1.20 | 1.10 |
| Microbial biomass | 1.14 | 1.04 |
| Fungi | 1.57 | 1.59 |
| Aerobic bacteria | 1.41 | 1.16 |
| Dehydrogenase | 1.50 | 1.40 |
| PMN[c] | 1.35 | 1.13 |

[a]Ratio of no-tillage to conventional tillage values.
[b]Ratio of Conservation Reserve Program to conventional tillage values.
[c]Potentially mineralizable nitrogen.

The practice of no tillage was developed to counteract the destructive effects of tillage. In two studies in the United States soil attributes from no-tillage fields were compared to those of conventionally tilled fields (Doran, 1980) and Conservation Reserve Program (CRP) fields were compared to paired, conventionally tilled fields (Staben *et al.*, 1997). The no-tilled fields were in no-till between 5 and 10 years and the CRP fields had been returned to grass for 7 years. The results are presented in Table 17.1 and are expressed as the ratio of the no-till data to the conventionally tilled or CRP data. Total soil C and N increased in the no-till soils but returning cropland to grassland had little effect. Similarly, the microbial biomass increased only in the no-till system compared to the conventional system. Returning fields to a less disturbed state significantly increased the numbers of fungi and bacteria and the dehydrogenase enzyme activity (a measure of biological activity). Nutrient cycling as measured as potentially mineralizable N increased by 35% in the no-till system and only 13% in the converted grass system. These data from widely different geographical locations and soil types show the importance and significance of tillage for organism populations and function. More importantly the CRP data suggest the effect on organisms is not simply due to increases in soil organic C.

In a compilation of 106 studies (Wardle, 1995), scientists were able to develop an index of the effects of tillage on detrital food webs and compare conventional tillage to no tillage. In most of the studies, the soil total C and N concentrations were less in the conventionally tilled soil compared to the no-tilled soil, as was the soil microbial biomass. However, as in the previous studies cited above, the increase in the microbial biomass in the no-till soils seemed to be greater than would be expected from an increase in SOM. This phenomenon is usually attributed to an increase in SOM quality due to frequent additions of residues to the surface of the

soil compared to rapidly decomposed buried residue, which contains more recalcitrant compounds.

In soil ecology, there is a good deal of interest in disturbance effects on trophic levels but also on organism diversity within the trophic level. A limited subset of studies tended to show that species diversity of the microfaunal groups is unchanged by tillage, while the macrofaunal groups can be elevated or reduced in diversity by tillage. The changes in organism biodiversity and its variability due to tillage are likely unpredictable because of the complexity and lack of understanding of the interactions between trophic levels. Overall, this indicates that soil food webs are fairly stable and have a significant amount of resiliency.

The microflora (bacteria and fungi) were generally mildly inhibited by tillage, whereas the microfauna of nematodes and protozoa was mildly to moderately inhibited by tillage in 50% of the studies. The mesofauna group of collembolan and mites tended to be moderately inhibited and the macrofauna group of earthworms and beetles was moderately to extremely inhibited by tillage. Thus, in general the larger organisms were more likely to be reduced than the smaller organisms under tillage systems compared to no-till systems.

Compaction is widespread worldwide and particularly significant where intensive mechanization is applied to soils subject to high rainfall or irrigation. Soil compaction has dramatic effects on plant growth, the soil biota, and biological processes. Compaction has been shown to affect specific microbial activities, e.g., soil respiration and the denitrification potential through direct effects on soil water content and aeration. A study (Robertson *et al*., 2000) showed that greenhouse gas emissions were eightfold higher in compacted conventionally tilled systems than in no-till. There is also increasing evidence for the role of pore size in regulating soil microbial populations and their biochemical processes. The shift in the relative abundance of pore sizes induced by soil compaction directly affects the abundance of microbial populations (Postma and van Veen, 1990). Compaction reduced the predation efficiency of protozoa or nematodes on bacteria due to a decrease in accessible pore space. The manipulation of habitable pore space due to tillage disturbance and soil compaction may regulate the types of organisms present and thus control the transformation of essential plant nutrients.

Tillage not only reduces SOM through disruption and oxidation, but also can create significant soil erosion over the landscape. The loss of soil through erosion is a significant problem worldwide. In some areas with highly erodible landscapes, such as areas of the United States, soil loss rates can exceed 38 T soil $ha^{-1}$ $year^{-1}$. It is estimated that about 20% of the soil C dislocated by erosion will be released into the atmosphere as $CO_2$, the rest being deposited over other land areas and into streams and rivers. Since it is mainly surface soil that is lost during erosion events, there is an associated loss of SOM high in biological activity. Toward the end of the 20th century, conservation tillage practices were increasingly implemented to reduce erosion and increase soil fertility. These practices have also been shown to increase soil biological activity and the mass of soil organisms in the surface soil.

Soil erosion and SOM loss are by-products of agricultural production and contribute to C and N losses. There is also a parallel and significant effect on soil organism populations and activity. Biological activity and biodiversity are important in determining deleterious effects of management on soil physical and chemical properties and the resulting effects on the sustainability of agricultural land. More research is needed to identify the relationships between microbial community composition (biodiversity), soil processes, and soil fertility on the health of the soil and its ability to provide a medium for plant growth.

## RANGELAND AND FOREST HEALTH

Much of the environmental movement of the 1970s and 1980s focused on decreasing the degradation of the environment, be it developing better fishing equipment to decrease damage to ocean bottoms and the trapping of nontarget fish to educational campaigns concerning the destruction of the rainforests. In the 1990s, the scientific community turned from monitoring degrading practices to evaluating a holistic attribute, ecosystem health. This direction began with work on soil quality, much like air and water quality assessments of the 1980s, and evolved into such work as rangeland health and forest ecosystem health. The National Research Council suggested that rangeland health be defined as "the degree to which the integrity of the soil and ecological processes of rangeland ecosystems are sustained." Healthy would be the dictionary definition of: (1) functioning properly or normally in its vital functions, (2) free from malfunction of any kind, and (3) productive of good of any kind. These definitions also apply to forest ecosystems.

At the core of ecosystem health is soil quality, defined as "the capacity of a soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health." The major issues are:

1. productivity—the ability of soil to enhance plant and biological productivity;
2. environmental quality—the ability of soil to attenuate environmental contaminants, pathogens, and offsite damage;
3. animal health—the interrelationship between soil quality and plant, animal, and human health.

Within the context of the soil quality concept, it is evident that soil biology, specifically microorganisms and their processes, will play a major role in determining the health of the ecosystem. Thus, *in situ* parameters or management that enhance or degrade microbial habitat will shift the health of the system. Attributes that affect the functioning of the soil system are listed in Table 17.2 and, when measured in the context of the proper ecological question, can be used to evaluate ecosystem health. The proper ecological question means "what is the function of the ecosystem?" or "what is the ecosystem going to be used for?" Thus, the soil quality attributes for an urban area may be different from the soil quality attributes for range- and pasture land.

**TABLE 17.2** Proposed Soil Physical, Chemical, and Biological Characteristics to Be Included as Basic Indicators of Soil Quality (Adapted from Doran and Parkin, 1996)

| Soil characteristic | Methodology |
|---|---|
| Physical | |
| Soil texture | Hydrometer method |
| Depth of soil and rooting | Soil coring or excavation |
| Soil bulk density and infiltration[a] | Field-determined using infiltration rings |
| Water-holding capacity[a] | Field-determined after irrigation of rings |
| Water retention | Water content at 33 and 1500 kPa tension |
| Water content[a] | Gravimetric analysis: wt loss, 24 h at 105°C |
| Soil temperature[a] | Dial thermometer or hand temperature probe |
| Chemical | |
| Total organic C and N | Wet or dry combustion, *volumetric basis*[b] |
| pH | Field- or lab-determined, pocket pH meter |
| Electrical conductivity | Field or lab, pocket conductivity meter |
| Mineral N ($NH_4$ and $NO_3$), P, and K | Field or lab analysis, *volumetric basis* |
| Biological | |
| Microbial biomass C and N | Chloroform fumigation/incubation, *volumetric basis* |
| Potentially mineralizable N | Anaerobic incubation, *volumetric basis* |
| Soil respiration | Field-measured using covered infiltration rings, lab-measured in biomass essay |
| Biomass C:total organic C ratio | Calculated from other measures |
| Respiration:biomass ratio | Calculated from other measures |

[a]Measurements taken simultaneously in field for varying management conditions, landscape locations, and time of year.

[b]Gravimetric results must be adjusted to volumetric basis using field-measured soil bulk density for meaningful interpretations.

A major attribute of healthy soil is the level of SOM, which controls many of the chemical and physical parameters of soil. For example, in Table 17.2 SOM can influence bulk density, water-holding capacity and retention, and soil temperature and buffer the soil pH and electrical conductivity as well as influencing biological activity. However, as we have seen SOM can be rapidly lost through oxidation and by wind and water erosion processes. Most of the 1965 million hectares (Mha) of degraded land worldwide suffers from low organic matter content. Thus, the single most overriding factor for increasing soil quality and ecosystem health is increasing the level of SOM.

Coupling the ecosystem health concept with increasing SOM levels has recently drawn attention due to global warming and greenhouse gas issues. Since the most abundant greenhouse gas is $CO_2$ and $CO_2$ is the raw material for SOM after it is fixed by plants, increasing SOM will remove $CO_2$ from the atmosphere. Significant scientific activity has been aimed at estimating the potential for soils to sequester more SOM and thus be a sink for atmospheric $CO_2$. An example would be to determine from the literature that soils of the Midwest have lost

upward of 50% of their SOM due to cultivation and then calculate how much C could be sequestered by returning this land to the native state. In reality, this is not a trivial calculation; however, this analysis can be done with many types of land and management practices (see suggested reading). Rangelands and forests have been identified as the ecosystems likely to store significant amounts of additional C in soil and plant material; however, it will be the soil microflora and their processing of plant C that will govern the rate and amount of C that is stored.

The magnitude of rangeland and forest land in the United States and worldwide is staggering. Grazing land in the United States constitutes 336 Mha, 48% of which is technically rangeland (161 Mha), about the same as cropland; rangeland combined with grassland pasture totals 239 Mha. Grazing land is estimated to cover 55% of the total U.S. land area and about the same on a global basis. Forest ecosystems occupy 298 Mha in the United States and 3650 Mha globally, excluding boreal forests. Ecosystem scientists believe that through proper management, these ecotypes occupying vast areas can sequester large amounts of C in the soil, providing an increase in soil quality and ecosystem health and removing the greenhouse gas $CO_2$ from the atmosphere.

There are a number of management practices and land use changes that could benefit increasing soil C in range- and grazing land. Much of the semiarid and arid grazing lands are highly erodible subject to both wind and water erosion and would benefit by conversion to managed grasslands with greater input intensity. Improving grazing management has been shown to increase soil C compared to nongrazed exclosures. However, the increases in total soil C and N with grazing may be soil texture dependent. The conversion of forest to pasture and marginal cropland to pasture can also substantially increase soil C as can better management of fertility and plant species composition. The estimations of the amount of soil C that could be stored in managed U.S. privately owned grazing lands range from 30 to 110 million metric tons (MMT) C year$^{-1}$ or 142 to 519 kg-C ha$^{-1}$ year$^{-1}$. The latter value can be compared to the CRP land that converts cropland to grassland and increases the sequestration of soil C by 400 to 1000 kg-C ha$^{-1}$ year$^{-1}$.

Forest management practices also contribute to the increase in soil quality and ecosystem health by increasing soil C. The conversion of marginal land and highly erodible land to forest will increase soil C and also provide an aboveground component of sequestered C to the ecosystem. Increasing intensively managed timberland and canopy cover in urban areas has a large potential effect on soil C and maximum C sequestration. Even with the negative aspects of wood removal and burning, growing short-rotation woody crops for energy has a substantial potential to sequester soil C. The U.S. estimates for these practices on forest and marginal land range from 276 to 529 MMT-C year$^{-1}$; this can be compared to an estimate of 75 to 208 MMT-C year$^{-1}$ from more intensive management of U.S. cropland.

The potential amount of increased C sequestration in soils of grazing lands and croplands of the United States amounts to about 13% of the 1600 MMT-CE year$^{-1}$ (CE represents C equivalents) greenhouse gas emissions from the United

States. With the increase in C sequestered by forests in soil and above ground this percentage more than doubles. Thus, soil C sequestration is a viable mechanism of reducing greenhouse emissions from the United States. Considering the global magnitude of the land area in rangeland and forest, if these lands could also be managed, soil C could be a significant strategy in reducing global greenhouse gases.

Thus when speaking about the health of an ecosystem, we must consider the whole system, including feedbacks. The system just described shows the benefit of increasing SOM to forest ecosystem health in addition to the benefit of reducing greenhouse gases. However, since increasing SOM is a balance between primary plant production and microbial decomposition it is the maintenance of suitable microbial habitat that will control the ecosystem health.

## ALTERNATIVE AGRICULTURAL MANAGEMENT

### ORGANIC AGRICULTURE

Organic agriculture strives to integrate human, environmental, and economically sustainable production systems. The term organic does not necessarily refer to the types of inputs to the system but more to the holistic interaction of the plants, soil, animals, and humans in the system. Organic agriculture management promotes maintaining SOM levels for soil fertility, providing plant nutrients through microbial decomposition of organic materials, and control of pests, disease, and weeds with crop rotations, natural control agents, and pest-resistant plant varieties (Oelhaf, 1978; Lampkin and Measures, 2001). Organic agriculture is practiced in almost all countries and has been applied to all agricultural products including livestock and milk.

In the 1990s, the land area under organic agriculture production and the number of agricultural products produced organically exploded worldwide. At the present time, there are 24 Mha worldwide in organic production, including grazing lands for organic livestock production. The global distribution of organic farmed land and the number of farms are shown in Fig. 17.1. The large land area and small number of farms in the Oceania/Australia region is due to large areas used for grazing livestock production with no inputs. Early in organic agriculture development, standards varied and certification of organic production was controlled on a regional basis or within country organizations. Now organic certification bodies worldwide are accredited by the International Federation of Organic Agriculture Movements (www.Ifoam.org), which sets basic standard organic practices.

Soil properties and soil functioning will be altered as a consequence of converting from conventional fertilizer-based farming to organic farming. During the conversion to organic production SOM levels and other nutrients can decrease (Wander *et al.*, 1994; Rees *et al.*, 2001). However, if manures are incorporated into the transition increases in these soil properties can be achieved. Transition affects

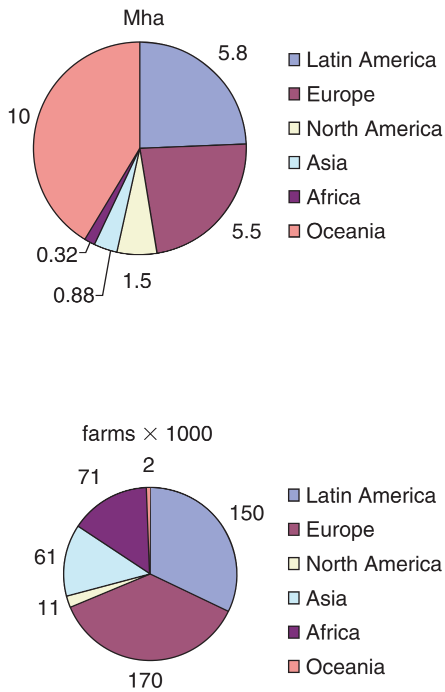



FIGURE 17.1 Hectares in organic food production and number of organic farms on a global basis.

a combination of changes in the microbial community and a physical redistribution of SOM fractions (Wander and Traina, 1996; Clark *et al.*, 1998). In the long term, soils under organic management will tend to increase in SOM. In addition, organically managed soils also have increased levels of biologically active SOM, which promotes an active microflora. This is why organically managed soils have a greater microbial biomass, more active microbial respiration, and a higher rate of N mineralization than conventionally farmed soils (Reganold *et al.*, 1993; Clark *et al.*, 1998).

Since organic systems are often low nutrient input systems, with respect to N, P, and K, the cycling of SOM by microorganisms is important because plants rely on nutrients solely from SOM. These systems may work due to an increase in microbial biodiversity as transition to organic management occurs. Organic systems have higher catabolic activity and nutrient cycling stability as the microbial diversity increases. However, this increase in diversity and its relationship to soil function are poorly understood, leading others to conclude that microbial biodiversity is not the primary regulator of SOM dynamics. The mechanistic basis of the microbial diversity–soil function relationship with respect to C and N cycling needs further study.

There are scant scientific studies on the nutritional benefits of organically grown foods; however, higher levels of vitamin C, flavonoids, and nutrient elements Ca, Mg, and Fe and superior taste are commonly cited. Comparatively, in conventionally fertilized soils it has been shown that excess N can decrease the nutritional quality and taste of crops and make plants more susceptible to diseases and insects. Excess N is also responsible for decreasing the vitamin C of crops. The most often cited benefits of organically grown foods are probably what may not be on and in the foodstuffs, such as herbicide and pesticide residues and high concentrations of nitrates. In addition, no genetically modified (GM) crops or ingredients are allowed in organic foods, and in animal production there is no routine use of antibiotics.

## BIODYNAMIC AGRICULTURE

Another example of organic-based food production is biodynamic agriculture or farming, which is rooted in the lectures of Dr. Rudolf Steiner in 1924 to a group of farmers concerned with decreasing crop quality, yield, and animal health. Dr. Steiner was a philosopher and editor of Goethe's scientific writings. The lectures described a holistic view of a farm as an organism with the plant and animal community of a natural habitat striving for a certain balance in which the numbers of species and individuals are constant. Farm management including clearing, plowing, cutting, and grazing along with using monoculture crops creates an imbalance in the system, preventing periods of recovery, thus creating an unsustainable agricultural system. In similar fashion the use of pesticides kills both pests and beneficial organisms, creating an imbalanced system, which destroys productivity. The biodynamic approach is based on an understanding of the interrelationships between of living organisms and the processes that make up the ecological system (Koepf *et al*., 1976).

Biodynamic farming is a system of organic farming that includes crop diversification, use of green manures, and use of compost and manures improved by biodynamic preparations. The biodynamic preparations consist of selected plant and animal substances that undergo fermentation for a year and then are used to enhance compost and manure used in the farming operation. These preparations can also be applied directly to soil as a spray to enhance biological activity. The use of biodynamic preparations is the main difference between biodynamic farming and traditional organic agriculture.

Part of the biodynamic philosophy is that a healthy, active soil microbial population will enhance plant–microbe interactions and nutrient cycling and reduce soil pathogens. Studies over the first decade of the 21st century have shown that biodynamically managed fields maintain higher soil C levels, microbial respiration, mineralizable N, earthworm populations, and microbial biomass C and N and greater enzyme activities. The biodynamic farms had better overall soil quality mostly due to enhanced microbial decomposition and stabilization of organic matter. Thus, the manipulation of soil organism populations to a larger, more active,

and perhaps more diverse community through particular amendments provides a means to shift from a chemical-based to an organic-based agriculture.

## COMPOSTING

Composting is the active process of converting organic material to more stabilized forms of C through the action of microorganisms. Specifically, composting is the biological decomposition of wastes consisting of organic substances of plant or animal origin under controlled conditions to a state sufficiently stable for storage and utilization (Diaz *et al.*, 1993). Compost as a product can be used in gardens, in nurseries, and on agricultural land. With respect to management of organisms, composting is perhaps the prime example since we manage the microbial process and the microbial product and manage the use of compost in microbially based systems (Cooperband, 2002). As the compost definition implies, practically any plant or animal material can be composted. Compost plays a major role in the agriculture of developing countries using organic agriculture and biodynamic farming, being relied upon to provide organic matter and nutrients and increase soil tilth. It also plays a role in processing the human waste stream. The United States alone produces nearly 10 MMT of sewage sludge and 185 MMT of garbage annually, on a dry weight basis. Less than 15% of municipal solid waste is recycled; however, more than 30% of the sewage sludge is beneficially used as composted products (Rynk, 1992; http://compost.css.cornell.edu/OnFarmHandbook).

Traditionally yard waste is thought of as "the" compost material; however, manure, meat and dairy waste, wood, sawdust, and crop residue can be composted. In addition, animal carcass composting is receiving significant attention due to the environmental benefits versus burial, which can contribute to groundwater contamination. One important aspect of the material that affects the compost process and product is the C:N ratio of the starting material, ideally it should be 25 to 30:1. Typical C:N ratios of different materials are shown in Table 17.3. Materials can be

TABLE 17.3 C:N Ratios of Various Compost Materials

| Materials | C:N |
|---|---|
| Activated sludge | 6 |
| Grass clippings | 12–15 |
| Manure | 20–50 |
| Poultry manure | 15 |
| Soil humus | 10 |
| Sawdust | 200–500 |
| Vegetable waste | 12 |
| Wheat straw | 80 |
| Wood | 400 |

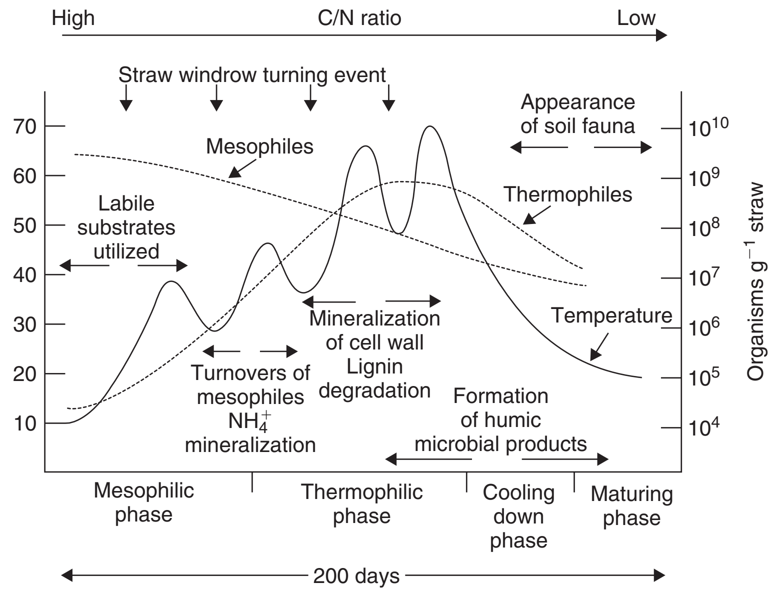



FIGURE 17.2 The organisms and processes occurring during composting of straw. The length of time varies with outside temperature and extent of mixing but usually involves 200 days (from W. R. Horwath, personal communication).

mixed to adjust the C:N ratio for a consistent product. There are numerous methods used for preparing materials and the environment for the composting process, including using waste materials alone, mixing organic materials of different quality, adding external nutrients and/or inocula, and controlling the physical environment to promote aerobic or anaerobic decomposition.

In compost terminology, process strategy refers to the management of the biological and chemical activity of the composting process. The biological processing uses terminology referring to the stage of the composting, such as active stage (mesophilic), high-rate stage (thermophilic), controlled (cooling), and curing stage (maturing) (Fig. 17.2). Configuration refers to the physical management of the process such as using piles, stacks, or windrows. Composting configurations range from windrow or open systems to enclosed systems, with windrow further classified as either static or turned. An example of a static system would be a stationary undisturbed mound of organic material with air being forced up through the mound or pulled down through the mound. In contrast, a turned system uses mixing as the aeration method, which also enhances the uniformity of decomposition and reduction in material particle size. The turned system is considered the traditional composting method for organic material (see Diaz *et al.*, 1993, for more detail). With any composting system managing the composting process will consistently produce compost with the desired characteristics.

The most prevalent composting technique is aerobic decomposition, carried out by a diverse microbial population that changes composition as conditions change. This method is preferred since it proceeds more rapidly and provides a greater reduction in pathogens and weed seeds because higher temperatures are achieved. Physiochemical factors affecting aerobic composting are temperature, moisture, aeration, pH, additives, particle size, and the C:N ratio of the composted substrate. Mostly indigenous organism populations are used for the composting process; microbial inoculants are utilized only under certain conditions. Figure 17.2 depicts the process of composting straw for 200 days under optimum conditions of temperature and moisture. In the mesophilic stage metabolism of the labile-C-rich substrates increases rapidly, generating heat. At this point there is a mixture of bacteria, actinomycetes, and fungi contributing to the decomposition process. In the early and transition stage to thermophilic conditions the windrow is turned, causing a decline in temperature and oxygenation of the inner material, resulting in rapid decomposition and temperature increase. As the temperature reaches 40°C the system turns from a mesophilic to a thermophilic stage, favoring mainly thermophilic bacteria and actinomycetes, with *Bacillus* being the dominant genus. Common *Bacillus* species found at this stage, and accounting for 10% of the decomposition, are *brevis*, *circulans*, *coagulans*, *licheniformis*, and *subtilis*. Decomposition will continue in the thermophilic zone until substrates begin to decline, then a gradual decrease in temperature will occur (Fig. 17.2). As the temperature declines the mesophilic organisms reappear, especially fungi that have preference for the remaining lignin and cellulose substrates. Fungi, responsible for 30 to 40% of the decomposition the compost material, include *Absidia*, *Mucor*, and *Allescheria* spp., *chaetomium*, *thermophilum*, *dactylomyces*. The actinomycetes, such as the *Norcardia* spp., *Streptomyces thermofuscus*, and *S. thermoviolaceus* are important in this phase when humic materials are formed from decomposition and condensation reactions. The actinomycetes are estimated to account for 15 to 30% of the decomposition of composted material.

The compost produced from this process is lower in C than the initial material, has a lower C:N and a higher pH, and can contain considerable $NO_3$. The end product of composting depends on the original substrate, any added nutrients, degree of maturity, and composting method; typical properties of composted plant material are listed in Table 17.4. Adding compost to soil increases the SOM, which increases soil structure and water-holding capacity and infiltration. In addition, compost contains significant amounts of plant nutrients such as N, P, K, and S and micronutrients, which are slowly released into the soil. As an ancillary benefit compost contains fairly resistant C compounds and may be dominated by fungi. Using compost on a garden or agricultural soil would favor an increase in the population of fungi and thus an increase in the fungi:bacteria ratio. Fungi are very abundant in soils and can constitute as much biomass as roots and as a group they are also the major organic matter decomposers in soil. Increasing the soil fungal population can increase C compounds that are agents in binding soil particles into aggregates, which increase soil tilth. Recent studies (Bailey *et al.*, 2002) have

TABLE 17.4 General Compost Properties

| | |
|---|---|
| %N | >2 |
| C:N | <20 |
| %Ash | 10–20 |
| %Moisture | 10–20 |
| %P | 0.15–1.5 |
| Color | Brown black |
| Odor | Earthy |
| %Water-holding capacity | 150–200 |
| CEC (meq 100 $g^{-1}$) | 75–100 |
| %Reducing sugars | <35 |

shown there is increased soil C storage in soils with greater fungal:bacterial ratios. Thus, as a consequence of using compost on our soil we have managed the soil microorganism population to our benefit.

## CROP ROTATIONS AND GREEN MANURES

Crop rotations have been practiced over the long history of agriculture. Studies dating from the 1840s on have shown that N supplied to grain crops was the major reason for using crop rotations containing legumes (Triplett and Mannering, 1978). With the advent of inexpensive nitrogen fertilizers, crop rotations containing legumes declined. Only recently has the value of crop rotations specifically including legumes been recognized as critical in maintaining SOM and soil productivity. Researchers in Canada studied the nutrient dynamics in a Canadian Luvisol after 50 years of cropping to a 2-year rotation (wheat–fallow) or a 5-year rotation (wheat–oats–barley–forage–forage) (McGill *et al*., 1986). Their results showed that the soil cropped to the 5-year rotation contained greater amounts of organic C and N. In addition they found that microbial turnover (i.e., carbon mineralization) was twice as fast in the 2-year rotation. The 5-year rotation doubled the input of carbon into the soil over the 2-year system and had a greater percentage of organic C and N in biological form. These results suggest that longer cropping system rotations that include forage or legumes will conserve SOM, maintain a greater biological nutrient pool, and put more nutrients into the soil than intensive rotations.

In a 10-year study, a low-input diverse crop system with manure and a low-input cash grain system with legumes showed significant increases in SOM compared to a conventional corn/soybean rotation (Wander *et al*., 1994). In addition, in both low-input (multiple crop rotations) systems the microbial biomass was greater and its activity higher than the conventional rotation of corn/soybeans with chemical inputs. The low-input systems also mineralized significantly more N and the microbial biomass contained 33 kg N $ha^{-1}$ more N than the conventional system.

In agricultural systems, plant pathogens are an important part of the soil microbial community. As growers reduce tillage and incorporate a greater variety of crops in rotation they face an increasing number of plant diseases that can cause significant stand and yield reductions. These potential losses, however, may be offset in systems incorporating green manures by promoting disease-suppressing properties that reduce plant pathogens, either (1) by increasing the levels of SOM that create conditions supporting a greater microbial biomass, competition for resources, antibiosis, or antagonism or (2) through direct inhibition by production of antibacterial/fungal compounds as in the case of *Brassica* cover crops that produce isothiocyanates. Cover crops are known to control disease-causing organisms through competition for resources and space, control of soil micronutrient status, and alteration of root growth.

## THE POTENTIAL FOR MANAGING MICROORGANISMS AND THEIR PROCESSES

### MANAGEMENT OF NATIVE AND INTRODUCED MICROORGANISMS

There are many cases in which soil organisms have been managed to improve plant growth and serve as biological control agents to suppress plant disease, inhibit weeds, control insects, or detoxify environmental pollutants (Table 17.5). Beneficial microorganisms directly promote plant growth through the creation of symbiotic associations with plant roots, release of phytohormones, induction of systemic resistance, suppression of pathogens, production of antibiotics, and reduction of heavy metal toxicity (Bowden and Rovira, 1999). Many of these organisms are naturally present in soil, although under some circumstances it may be necessary to increase their populations either by modifying the soil environment or through inoculation to enhance their abundance and activity.

The positive role of symbiotic associations such as mycorrhiza in plant production is well known (see Chap. 10), with many cases documenting growth and yield enhancement of infected plants. The plant response is due to an increase in effective root area that improves water and nutrient extraction. Other benefits of the association are protection against pathogens, improved tolerance to pollutants, and greater resistance to water stress, high temperatures, and adverse pH. Management strategies that enhance mycorrhizal populations and activity in agricultural fields include reduced tillage, crop rotation, and lower N and P applications. The other major plant symbionts, rhizobia associated with legumes, provide the N required for plant growth and in return obtain photosynthetic products from the plant for their own growth.

Many bacterial species have been used as plant-growth-promoting organisms, predominately pseudomonads (e.g., *Pseudomonas fluorescens*, *P. putida*, *P. gladioli*),

**TABLE 17.5** Examples of Bacteria and Fungi Introduced to Improve Agricultural Productivity, Control Pests, and Degrade Toxic Substances in Soil

| Example | Microorganisms |
|---|---|
| Plant growth | *Bradyrhizobia*, *Mycorrhizae*, *Azotobacter*, PGPRB[a] |
| Biological control | |
| Plant pathogens | *Trichoderma*, *Fusaria*, *Pseudomonas* |
| Insects | *Bacillus*, *Saccharopolyspora* |
| Weeds | *Colletotrichum*, *Phytophthora*, *Sclerotinia*, *Pseudomonas* |
| Bioremediation | |
| Aromatic hydrocarbons | |
| Polycyclic | *Stropharia*, *Sphungomonas* |
| BTEX (benzene, toluene, ethylbenzene, xylene) | *Bacillus*, *Cladophidophora*, *Pseudomonas*, *Azoarcus* |
| Pesticides–carbamates | *Achromobacter*, *Pseudomonas* |
| Organophosphates | *Flavobacterium*, *Pseudomonas* |
| Pentachorophenol | *Athrobacter*, *Mycobacterium*, *Flavobacterium*, *Phanerochaete* |
| Triazines | *Klebsiella*, *Rhodococcus* |
| Heavy metals, radionuclides | *Deinococcus*, *Geobacter*, *Shewanella* |

[a]PGPRB, plant-growth-promoting bacteria.

bacilli (e.g., *Bacillus subtilis*, *B. cereus*, *B. circulans*), and others (e.g., *Azospirillum*, *Serratia*, *Flavobacterium*, *Alcaligenes*, *Klebsiella*, *Enterobacter*). Many of these bacteria produce the growth regulators auxins, cytokinins, gibberellins, or ethylene or abscisic acid, which stimulates plant growth (Arshad and Frankenberger, 1998). *Azospirillum* inoculation increases the density and length of root hairs as well as the elongation of lateral roots, increasing root surface area. The increased root area results in greater niche areas promoting greater colonization of species.

## MANAGING MICROBIAL POPULATIONS AS AGENTS OF BIOLOGICAL CONTROL

One of the fastest growing areas in pesticide development is the use of microorganisms for the biological control of pests (biopesticides). The fundamental concept of biological control is the selection and introduction of specific organisms to control a particular pest. The organism(s) selected might be a predator, parasite, competitor, or pathogen of the targeted pest. Biological control occurs widely in nature, masking the fact that most pests never become serious problems because of the controls exerted by other members of the community. Biological control methods are commonly a part of an overall integrated pest management program to reduce the legal, environmental, and public safety hazards of chemicals. In addition, it may be a less expensive alternative to the use of some pesticides. Unlike most pesticides, biological control agents are often specific for a particular

pest. Successful use of biological control requires a greater understanding of the biology of both the biocontrol agent and the targeted pest. Often, the outcome of using biological control is not as dramatic or quick as the use of pesticides. Most biocontrol agents attack only specific types of plant pathogens, nematodes, or insects unlike broad-spectrum pesticides, which kill a wide range of organisms.

There are three strategies by which organisms are managed to control pest populations. *Classical biological control* involves collecting organisms pathogenic to the pest from locations where a pest originated and releasing them in an infected area to control the pest. The natural increase in the biocontrol organisms is relied upon to control the pest without future intervention. *Augmentation* is a method of increasing the population of a natural enemy that attacks a pest. This can be done by mass producing inoculum in the laboratory and releasing it into the field at the proper time. Control results from and requires the increase of the antagonistic disease through many disease cycles to reach threshold levels that cause death or control of the target pest. The augmentation method relies upon continual human management. *Conservation* or enhancement of indigenous populations is an important part in any biological control effort. This involves identifying any factors that limit the effectiveness of a particular population and facilitate them to help the beneficial species. Conservation of natural enemies involves either reducing factors that interfere with the populations of natural enemies or providing needed resources that help them.

Many bacteria and fungal genera have been used extensively for the control of plant pathogens. Various nonpathogenic fungal strains of *Rhizoctonia*, *Phialophora*, *Fusarium*, and *Trichoderma*, as well as mycorrhizal fungi, have been used to reduce damage caused by related strains or other pathogenic fungi. As biocontrol agents, *Trichoderma* species dominate, most likely because of their ease of culture and wide host range. The most commonly targeted pathogens are *Pythium*, *Fusarium*, and *Rhizoctonia* species, reflecting their worldwide importance and their relative ease of control (Whipps, 2001). The most commercially successful bacterial-based biocontrol system is the use of the nonpathogenic *Agrobacterium* strains to control crown gall.

The primary modes of action of pathogen control or management of both bacteria and fungi are competition for C, N, and Fe and increased colonization of the rhizosphere by the nonpathogenic strains (Table 17.6). For more information on competition and food web interactions (see Chap. 8). Other mechanisms include antibiosis, induced resistance, and mycoparasitism. Competition for commonly used substrates and for their excretion sites on roots plays an important role in the root zone for controlling pathogens. Organisms capable of rapid growth rates with wide distribution and population numbers throughout the soil have a distinct advantage of colonization and substrate utilization that outcompetes many pathogens. Ectomycorrhizal fungi because of their physical sheathing morphology may occupy infection sites, thus excluding pathogens. In contrast, arbuscular mycorrhizas provide control of pathogens through induced resistance and improved plant growth rather than niche competition.

**TABLE 17.6** Mechanisms of Biocontrol by Soil Bacterial and Fungal Agents

| Method of control | Organism | Mechanism |
|---|---|---|
| Competition | | Niche exclusion, high reproduction rates, nutrient uptake, iron-chelating compounds |
| Antibiosis | | |
| Bacteria | *Pseudomonas*, *Bacillus*, *Streptomyces*, *Agrobacterium* | Inhibition by production of antimicrobial compounds, e.g., HCN, oomycin A, 2,4-diacetylphoroglucinol, zwittermycin, phenazine 1-carboxylic acid, pyrrolnitrin, agrocin 84 |
| Fungi | *Trichoderma*, *Talaromyces*, *Penicillium* | Inhibition by production of antimicrobial compounds, e.g., glioviron, gliotoxin, hydrogen peroxide, penicillin |
| Induced resistance | *Bacillus*, *Pseudomonas*, *Fusarium*, *Trichoderma*, *Pythium* | Biochemical changes in plant cell walls that are induced by physical or biochemical agents, e.g., siderophores, chitinases, peroxidase, lipopolysaccharides, phytoalexins, ethylene, salicylic acid |
| Mycoparasitism | *Verticillium*, *Trichoderma*, *Coniothyrium* | The process of mycoparasites is to sense host, direct growth, contact, recognize, attach, penetrate, and exit; they produce cell-wall-degrading lytic enzymes, chitinases, β1,3-glucanases, cellulases, proteases |

The production of antimicrobial metabolites is one of the most studied aspects of biocontrol. Antibiotics produced by a wide range of bacteria and fungi include pyrrolnitrin, phycocyanin, 2,4-diacetylphoroglucinol xanthobactin, and zwittermycin A.

## CONTROL OF INSECTS

Insect infestations can cause a significant decrease in crop productivity. Infestations have been historically difficult to control without the use of highly toxic synthetic chemicals. The most well known biopesticides for insect control are the *Bacillus thuringiensis* (*Bt*) formulations for the control of lepidopterous pests. This bacterium produces a protein that by itself is harmless to most insects, but is converted to a potent toxin in the gut of specific target insects following ingestion. The mode of action of *Bt* is very specific. Different strains of *Bt* are specific to different receptors in the gut wall of insects. *Bt* toxicity depends on recognizing

receptors; damage to the gut by the toxin occurs upon binding to the receptor. Each insect species possesses different types of receptors that will match only certain toxin proteins. Application of particular *Bt* toxin proteins has to be carefully matched to the target pest species. This is also an advantage to beneficial insects since they will not be adversely affected by select strains of *Bt*.

### BOX 17.1 History of Discovery: *Bacillus thuringiensis* (*Bt*)

In 1901, a Japanese biologist, Shigetane Ishiwatari, studying the cause of sotto disease that was devastating silkworm populations first isolated the bacterium *B. thuringiensis* (*Bt*) as the cause of the disease, but at that time named it *Bacillus sotto*. In 1911, Ernst Berliner isolated a bacterium that had killed a Mediterranean flour moth and named it *B. thuringiensis*. In 1915, Berliner reported the existence of a crystal within *Bt*, but the function of the crystal was not determined until 1956. In 1956, Hannay, Fitz-James, and Angus found that the primary insecticidal activity against lepidoteran insects was due to the parasporal crystal. Following this discovery research was begun to determine the crystal structure, biochemistry, and general mode of action of *Bt.* In the United States, *Bt* was used commercially starting in 1958. By 1961, *Bt* was registered as a pesticide by the Environmental Protection Agency.

By 1977, 13 *Bt* strains had been described. All were toxic to specific species of lepidopteran larvae. In 1977 the first subspecies toxic to dipteran species was described, and the first discovery of strains toxic to species of coleopteran were discovered in 1983. In the 1980s, use of *Bt* increased when insects became increasingly resistant to synthetic insecticides.

With advancements in molecular biology, it became possible to move the gene that encodes the toxin into plants. The first genetically engineered plant, corn, was registered by the EPA in 1995. Since 1996 plants have been modified with short sequences of *Bt* genes to express the crystal protein. With this method, transgenic plants produce the proteins and protect themselves from insects. Genetically modified crops, including potato and cotton, are planted throughout the world. In 1999, 29 million acres of *Bt* corn, potato, and cotton were grown globally. Today, there are thousands of strains of *Bt*. Many of them have genes that encode unique toxic crystals in their DNA.

#### POTENTIAL RISKS TO USING *BT*

- Invasiveness—genetic modifications, through traditional breeding or by genetic engineering, can potentially change the organism to become invasive. Few introduced organisms become invasive, although it is a concern for the users.

- Resistance to *Bt*—the biggest potential risk to using *Bt* crops is resistance.
- Cross-contamination of genes—although unproven, genes from genetically modified crops can potentially introduce the new genes to native species.
- For these reasons there are numerous societal concerns about the use of genetically engineered plants, including those with *Bt* insertions.

Adapted from "History of *Bt*," Dr. Raffi V. Aroian, University of California at San Diego, La Jolla, CA, USA: http://www.bt.ucsd.edu/bt_history.html.

The gene coding for this protein has been cloned from various *B. thuringiensis* strains and has been incorporated into the genome of a number of plants (corn, cotton, potato). The insect against which the toxin is active dies soon after feeding on the transformed plant. A drawback to this approach is that resistance of the insect to the toxin may develop. The major advantage of the *Bt* toxin is that it is harmful to only a few species of insects, while it is harmless to other animals and humans. These biological pesticides also degrade rapidly in the environment. Thus, the use of such biological pesticides appears to be an environmentally safe alternative to pest control than the use of synthetic chemical pesticides.

Other examples of biocontrol agents of insects include the fungus *Metarrhizium anisopliae*, used to control larvae of grass grubs (*Costelytra*) in pastures, as well as several genera of nematode-trapping or nematophagous fungi (*Arthrobotrys*, *Dactylella*, *Verticillium*). Entomopathogenic nematodes of the *Delanus*, *Neoaplecantana*, *Tetradonema*, and *Heterorhabditis* have been found to control a wide range of insect pests. The success of these nematodes lies in the fact that most plant insect pests spend a part of their life cycle in soil.

## WEED CONTROL

Biological control of weeds has been studied for over 100 years. The approach taken has been to use fungal plant pathogens to control weeds by postemergence application of the plant pathogen to weed foliage. The most commonly studied fungi are *Colletotrichum*, *Phytophthora*, *Sclerotinia*, and *Puccinia*. For an in-depth discussion on the use of microbial pesticide control of weeds, see TeBeest (1996).

Although many of the organisms discussed have shown excellent efficacy in pure culture or microcosm studies, their adoption for wide use in agriculture is minor (except for *Bt*). This has been due to strict environmental regulations regarding their release and the technical problems associated with introducing and maintaining populations in the soil. Technical problems include: (1) identification of factors that affect survival rates, (2) determining which strains are best for each crop of interest, and (3) field conditions, methods of application, and implementation of management practices that enhance biocontrol.

Significant disease suppression and alteration of soil microbial populations can also be achieved by manipulating the physiochemical and microbiological environment through management practices such as soil amendments, crop rotations, tillage (as discussed earlier), natural or synthetic compounds (soil fumigation), soil solarization, or the use of genetically resistant varieties. Soils that are suppressive to pathogens typically have neutral to alkaline pH, and liming of acid disease-prone soils has been shown to reduce the severity of fungal pathogens.

## USE OF SYNTHETIC AND NATURAL COMPOUNDS TO MODIFY SOIL COMMUNITIES OR FUNCTIONS

The use of synthetic compounds to control pests began in the 1930s and became more widespread after the end of World War II. "First-generation" pesticides were largely highly toxic compounds, such as arsenic and hydrogen cyanide. Their use was largely abandoned because they were ineffective or too toxic. The second-generation pesticides largely included synthetic organic compounds. From about 1945 to 1965, organochlorines were used extensively in all aspects of agriculture and forestry, in protecting wooden buildings and protecting humans from a wide variety of insect pests. In recent years, chemical pesticides have become the most important consciously applied form of pest management. For crops in some areas, alternative forms of pest control are still heavily used, such as burning, rotation, or tillage. The wide use of synthetic pesticides (fungicides, bactericides, nematicides, insecticides, herbicides) dominates high-input production agriculture and forestry. In intensive cropping systems, plant pathogens can reach epidemic populations, endangering crop yields and quality.

*Soil fumigation* is aimed at controlling pests or at least reducing infestations to levels enabling successful growth of commercial crops (Ramsay *et al.*, 1992). Soil fumigants are chemicals (may be solids, liquids, or gases) that, when applied to soil under specified temperature and moisture conditions, generate toxic gases that can kill many kinds of pest organisms as the fumes spread through the soil. Multipurpose soil fumigants used to provide plant disease and nematode control include chloropicrin, dichloropropene, metam-sodium, metam-potassium, and methyl bromide. The latter will be phased out because it has been identified as an ozone-depleting substance. At the rates normally used to reduce populations of nematodes and many weeds and soilborne fungi, fumigants can also kill plants, so they are applied to the planting site weeks before planting. Fumigation does not stimulate plant growth directly; it only reduces numbers of nematodes and other pests for a limited period, during which the plants can produce healthy roots if no other growth-limiting factors are present. Fumigants have no residual effect, so nematodes or pathogens that survive or escape treatment (e.g., are too deep to be reached by the fumigant or are protected inside root tissues and soil aggregates) or are brought in after fumigation can begin to reinfest the root zone immediately. Many synthetic soil fumigants are general biocides that do not target specific genera or pathogens. Therefore, the activity of many soil fumigants has shown negative

effects on both beneficial and deleterious populations of soil organisms. Numerous studies show significant reductions in soil processes involved in the cycling of plant nutrients, symbiosis, and soil tilth.

Organic pesticides are usually considered as those pesticides that come from natural sources. The fundamental difference between organic and synthetic pesticides is not their toxicity, but their origin—whether they are extracted from natural plants, insects, or mineral ores or are chemically synthesized. These natural sources are usually plants, as is the case with pyrethrum (pyrethins), rotenone, or ryania (botanical insecticides), or minerals, such as boric acid, cryolite, or diatomaceous earth. Two of the most common organic pesticides, copper and sulfur, are used as fungicides by organic growers. Because they are not as effective as their synthetic counterparts, they are applied at significantly higher rates. Copper fungicides are used to treat foliage, seeds, wood, fabric, and leather as a protectant against blights, downy mildews, and rusts. In agriculture, copper compounds, especially copper sulfate, are used as fungicides, pesticides, algicides, nutritional supplements in animal feeds, and fertilizers. Copper is the 18th most used pesticide in the United States. Over 13 million pounds of copper were applied to 54 crops in 1997. Copper, oil, and sulfur combined accounted for a full 25% of U.S. pesticide use.

Organic pesticides are largely insecticides and nematicides, although they do have efficacy against some bacterial and fungal pathogens. The efficiency of oilseed meals of neem (*Azadirachta indica*), castor (*Ricinus communis*), mustard (*Brassica campestris*), and duan (*Eruca sativa*) has been evaluated against plant-parasitic nematodes and soil-inhabiting fungi that infest a number of crops. The population of plant-parasitic nematodes, *Meloidogyne incognita*, *Rotylenchulus reniformis*, *Tylenchorhynchus brassicae*, *Helicotylenchus indicus*, etc., and the frequency of the pathogenic fungi *Macrophomina phaseolina*, *Rhizoctonia solani*, *Phyllosticta phaseolina*, *Fusarium oxysporum f. ciceri*, etc., have been significantly reduced by these treatments; whereas populations of saprophytic fungi have increased.

*Soil solarization* is a nonchemical technique used for the control of many soilborne pathogens and pests (Katan and Devay, 1991; Gamliel *et al*., 2000). This simple technique captures radiant heat energy from the sun, thereby causing physical, chemical, and biological changes in the soil. Transparent polyethylene plastic placed on moist soil during the hot summer months increases soil temperatures to levels (71°C) lethal to many soilborne plant pathogens, weed seeds, and seedlings (including parasitic seed plants); nematodes; and some soil-residing mites. Soil solarization also improves plant nutrition by increasing the availability of nitrogen and other essential nutrients. Beneficial microbial populations, such as mycorrhizal fungi, *Trichoderma* sp., actinomycetes, and some beneficial bacteria survive the solarization process and rapidly recolonize the soil. These is turn may contribute to a biological control of pathogens and pests and/or stimulate plant growth. Soil solarization also has been shown to be effective at reducing nematode populations, but less dramatically than it does fungal pathogens and weeds.

Nematodes generally are more tolerant of heat and control is less effective in soil depths beyond 12 in. Solarization may therefore be useful and economically feasible for shallow-rooted crops.

## MANIPULATING SOIL POPULATIONS FOR BIOREMEDIATION OF XENOBIOTICS

Bioremediation manages microorganisms to reduce, eliminate, contain, or transform contaminants present in soils, sediments, water, or air. Various forms of bioremediation have been documented throughout recorded history. The composting of agricultural residues and sewage treatment are based on the use of microorganisms to catalyze chemical transformations. Composting dates back to 6000 BCE, with the modern use of bioremediation beginning over 100 years ago with the design and operation of the first biological sewage treatment plant in Sussex, England, in 1891. Over the past several decades, *in situ* degradation of biologically foreign chemical compounds (solvents, explosives, polycyclic aromatic hydrocarbons, heavy metals, radionuclides, etc.) has been used as a cost-effective alternative to incineration or burial in landfills (Alexander, 1994). An advantage of bioremediation over other methods is that it transforms contaminants instead of simply moving them from one source to another as in the practice of land filling (Table 17.7). Also it is relatively low cost compared to other methods of removal. In a bioremediation process, microorganisms break down contaminants to obtain chemical energy. It involves the manipulation of microorganisms and their metabolic processes (enzymes) to degrade compounds of concern. Figure 17.3 shows the microbial degradation pathway of a pesticide, DDT (Bumpus and Aust, 1987).

*Phanerochaete chrysosporium* (and several other species) uses a peroxidase enzyme system that acts in concert with $H_2O_2$, produced by the fungus, to degrade many recalcitrant organics, especially those with structures similar to lignin, which naturally degrades in soil systems. Degradable contaminants include DDT, lindane, chlordane, TNT, and PCBs. Bacteria and fungi have been shown to break down practically all hydrocarbon contamination in the natural environment.

Through studying natural processes, researchers have been able to determine what conditions are necessary for degradation and what organisms are active degraders of specific pollutants and manipulate them. Examples of aerobic bacteria managed for their degradative abilities include *Pseudomonas*, *Alcaligenes*, *Sphingomonas*, *Rhodococcus*, and *Mycobacterium*. These bacteria have been reported to degrade pesticides and hydrocarbons, both alkane and polyaromatic compounds (PAHs). Many of these organisms are capable of using the contaminant as the sole source of C and energy. In the absence of $O_2$, anaerobic bacteria have been used in the remediation of polychlorinated biphenyls (PCBs) and dechlorination of trichloroethylene (TCE) and chloroform. Ligninolytic fungi, such as P. *chrysosporium*, have the ability to degrade an extremely diverse group of persistent PAHs. Methylotrophs use the methane monooxygenase pathway to degrade a wide range of compounds including the chlorinated aliphatics, trichloroethylene,

TABLE 17.7 Description of Common Bioremediation Approaches

| | |
|---|---|
| *In situ* bioremediation of soil | The goal of aerobic *in situ* bioremediation is to supply oxygen and nutrients to the microorganisms in the soil and does not require excavation or removal of contaminated soils. *In situ* techniques can vary in the way they supply oxygen to the organisms that degrade the contaminants. Two such methods are bioventing and injection of hydrogen peroxide. Remediation can take years to reach cleanup goals. |
| Bioventing | Bioventing systems deliver air from the atmosphere into the soil above the water table through injection wells where contamination is located. Nutrients, nitrogen, and phosphorus may be added to increase the growth rate of the microorganisms. |
| Injection of hydrogen peroxide | This process delivers oxygen by circulating hydrogen peroxide through contaminated soils to stimulate the activity of indigenous microbial populations. |
| *Ex situ* bioremediation of soil | *Ex situ* techniques require excavation and treatment of the contaminated soil. *Ex situ* techniques include slurry- and solid-phase techniques. |
| Liquid slurry phase | Contaminated soil is combined with water and other additives in a bioreactor and mixed to keep indigenous microorganisms in contact with the contaminants. |
| Solid phase | Solid-phase bioremediation treats soils in aboveground treatment areas equipped with collection systems to prevent contaminants from escaping. |
| Landfarming | Contaminated soils are excavated and spread on a pad with a system that collects leachates or contaminated liquids that seep out of the contaminated soil. The soil is periodically turned to mix air or provide nutrients. |
| Soil biopiles | Contaminated soil is piled to several meters over an air distribution system. Moisture and nutrient levels are incorporated to maximize microbial activity. |
| Composting | Biodegradable contaminates are mixed with straw, hay, or other C-rich compounds to facilitate optimum levels of air and water to microbial populations. The three commonly used designs are static pile composting, mechanically agitated in-vessel composting, and windrow composting. |

Adapted from U.S. EPA (1996).

and 1,2-dichloroethane. *Geobacter sulfurreducens* (Fig. 17.4) grows with insoluble Mn(IV) oxides as the electron acceptor.

Several factors influence the success of bioremediation and should be considered on a site-by-site basis. Factors include the existence of a microbial population capable of degrading the contaminant, availability of contaminants in the microbial populations, the type of contaminant, its concentration, and environmental factors such as soil type, temperature, pH, the presence of oxygen, or other electron

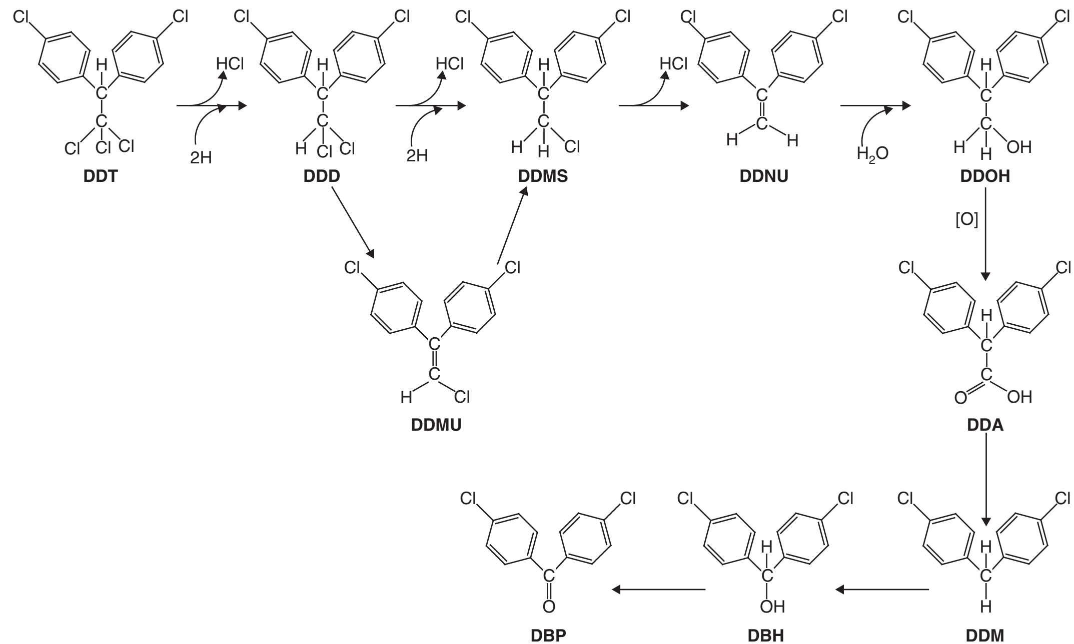



FIGURE 17.3 Proposed pathway for bacterial metabolism of DDT via reductive dechlorination. DDT, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; DDD, 1,1-dichloro-2,2-bis(chlorophenyl)ethane; DDMU, 1-chloro-2,2-bis(*p*-chlorophenyl)ethylene; DDMS, 1-chloro-2,2-bis(*p*-chlorophenyl)ethane; DDNU, 2,2-bis (*p*-chlorophenyl)ethylene; DDOH, 2,2-bis(*p*-chlorophenyl) ethanol; DDA, bis(*p*-chlorophenyl)acetic acid; DDM, bis(*p*-chlorophenyl)methane; DBH, 4,4′-dichlorobenzhyrol; and DBP, 4,4′-dichlorobenzophenone (with permission from Aislabie *et al*., 1997).

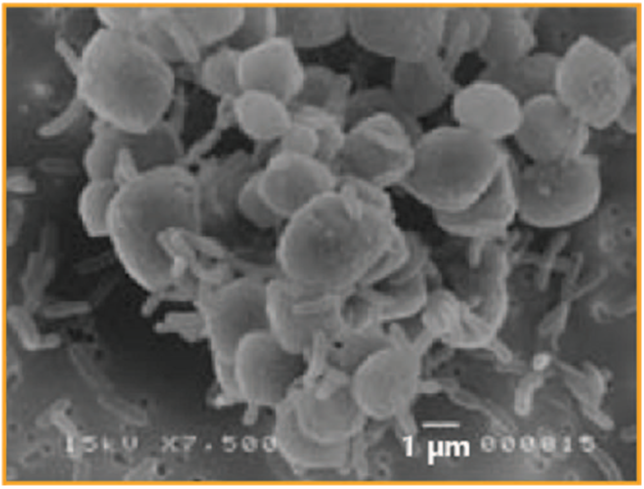



FIGURE 17.4 *Geobacter sulfurreducens* is the dominant metal-reducing microorganism isolated from subsurface environments and aquifers. It oxidizes aromatic hydrocarbons with Fe(III) as the electron acceptor. It can also oxidize organic pollutants while reducing other metals such as Mn(IV), U(VI), Te(VII), Co(VI), and Au(III). (Image courtesy of D. Lovely, University of Massachusetts. Reproduced from Anonymous (2003.)

TABLE 17.8 Environmental Conditions Influencing Biodegradation

| Parameter | Condition required for microbial activity | Optimum for oil degradation |
|---|---|---|
| Soil moisture | 25–28% of water holding capacity | 30–90% |
| Soil pH | 5.5–5.8 | 6.5–8.0 |
| Oxygen content | Aerobic, minimum air-filled pore space 10% | 10–40% |
| Nutrient content | N and P | C:N:P = 100:10:1 |
| Temperature (°C) | 15–45 | 20–30 |
| Contaminants | Not too toxic | Hydrocarbon 5–10% of dry wt of soil |
| Heavy metals | <2000 ppm | 700 ppm |
| Soil type | Low clay or silt content | |

From Vidali (2001).

acceptors and nutrients. Although microorganisms are present in contaminated soils, they are not necessarily present in the numbers required for intrinsic bioremediation. Their growth and activity are typically stimulated with the addition of nutrients and $O_2$ (Table 17.8).

The most common pollutants in the environment are the monoaromatic hydrocarbons produced from gasoline and diesel fuel production (Phelps and Young, 2000). Gasoline contains 20% by volume benzene, toluene, and xylene (BTEX). BTEX compounds enter the environment through accidental surface spills or

TABLE 17.9 Examples of Superfund Sites Using Bioremediation Technologies

| Site | Treatment | Contaminants |
|---|---|---|
| Applied Environmental Services, NY | Bioventing | Volatile organic compounds (VOCs), semivolatile organic compounds (SVOCs) |
| Onalaska Municipal Landfill, WI | Bioventing | VOCs, polyaromatic hydrocarbons (PAHs) |
| Eielson Air Force Base, AK | Bioventing | VOCs, SVOCs, PAHs |
| Brown Wood Preserving, FL | Land treatment | PAHs |
| Vogel Paint & Wax, IA | Land treatment | VOCs |
| Broderick Wood Products, CO | Land treatment/ bioventing | SVOCs, PAHs, dioxins |
| Burlington Northern (Somers), MT | Land treatment | SVOCs, PAHs |

From U.S. EPA (1996).

leakage of underground storage tanks or are added directly to agricultural soils as the "inactive" ingredients of pesticides. Many pesticide formulations contain 7–14% BTEX by weight as carriers. Benzene in particular is a known carcinogen and is soluble in water at concentrations far greater than the drinking water standard ($1\,\mu g\ liter^{-1}$). For BTEX compounds, the principal concern is their ready migration away from source areas.

Polycyclic aromatic hydrocarbons are the contaminants of concern at manufacturing petroleum plants and EPA Superfund sites across the United States (Table 17.9). Of the PAHs, the high-molecular-weight compounds (those with four or more rings) are of most concern with respect to health risks since a number of these compounds are known carcinogens. PAHs in general are characterized by very low solubility in water.

In addition, these compounds readily form nonaqueous phases in soil and sediment and also bind tightly to soil constituents such as SOM. Despite the limited water solubility of PAHs, PAH-contaminated sediment can lead to teratogenicity and toxicity of the water. Contaminated sediments represent a continuing source of contamination in the aquatic food chain.

Despite the high potential for bioremediation as an effective technology, its use is limited by the depth of understanding of biodegradation processes and inexperience in managing these processes in the field. This includes aspects of cometabolism, inoculation, evolution of biodegradation capabilities, monitoring and process control, measures of effectiveness, and genetic engineering.

## CONCLUDING COMMENTS ON MICROBIAL ECOLOGY

From the foregoing discussion it is evident that humans can and have managed microorganisms for our own benefit. However, much of our discussion has centered

on "unnatural" systems in which we manipulate the organism environment to accomplish the desired outcome. For example, culturing all of the organisms in a gram of soil and using them in a fermentation of grape juice would produce undrinkable wine, but inoculating the fermentation with specific species of microorganisms produces a quality product. In composting for gardens or commercial production, the operation is usually controlled at least with respect to ingredients, aeration, and moisture.

But what are the options and benefits with organisms and their management in "natural" environments? In natural environments, the mass of organisms and species composition (structure) are governed by the environment, including habitat, moisture, and temperature. Organisms form trophic levels with various modes of interaction and coexistence, from symbiotic to predatory. The community of soil organisms may increase or decrease and their composition may change principally due to influences of abiotic microclimate and natural input of metabolically available substrates (plant litter, leachates, and exudates). The complexity of the system dictates that introduced organisms must find a niche to survive and be persistent. In general, organisms introduced into a soil system do not usually survive more than a period of days to weeks. The reason for poor survival rates could be due to nutrient competition, susceptibility to predation and chemical attack due to differences in cell chemistry from culturing, or simply nutrient deficiencies.

In natural systems, organisms can be structured compartmentally to be close to other organism for symbiosis, away from other organisms for protection, and in proximity to nutrients and water. An example of organism symbiosis is fungi breaking down the macromolecule cellulose into smaller more "digestible" compounds that can be utilized by other bacteria and fungi that cannot utilize cellulose directly. In addition, an organism may need a specific growth factor or vitamin that may be produced by specific bacteria, thus by growing in a mixed culture (soil) the first organism receives "nutritional symbiosis." There is, however, a down side to living among a myriad of different organisms, that is, some are predators and some will resort to predation to survive. Predation is probably the biggest factor in organism composition changes over time. When carbon inputs from litter reach the soil, bacteria increase in numbers, and then bacterial-feeding protozoa increase and a new short equilibrium will be reached in community composition. This cyclic flux in population structure drives the complex food web system of the soil and dictates nutrient availability to plants. From this perspective, perhaps it is the soil microflora who is doing the "managing" in many ecosystems.

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# 18

# Soil Microbiology, Ecology, and Biochemistry for the 21st Century

Josh Schimel



> A book on soil microbiology should include a study of the occurrence of microorganisms in the soil, their activities and their role in soil processes. It is this last phase which has been studied least and where the information available is far from satisfactory in explaining what is taking place in the soil. —Selman Waksman, 1926 (Waksman, 1932).

## INTRODUCTION

The first chapter of this book discussed the history of soil biology, weaving the threads of microbiology, biochemistry, and ecology as they developed toward the

modern day. Many of our basic ideas about soil biology, such as N cycling processes, the concept of "active" vs recalcitrant litter fractions, and the concept of the links between microbial communities and processes were developed, though in a somewhat nascent form, by around 1930 (Waksman 1927, 1932). Over the decades since, those ideas have developed into much more sophisticated forms (Chaps. 2–17). We have developed a more "systems" view of the soil; for example, the idea of element cycles was not clearly developed in Waksman (1932), though all the components of the N cycle were in place. The concept of soil as an integral part of the ecosystem was also not well developed. The term "ecosystem" in the English language was coined in 1935 by Tansley (1935). The European literature had earlier developed a more ecosystem-oriented view of soil processes as shown by the further interpretations of Jenny (1941). One element of the growing ecosystem focus has been the shifting of focus away from agriculture to other kinds of human-impacted systems as well as to natural ecosystems (Chap. 1). We are developing a much stronger understanding of the role of soil food webs and consumer dynamics in soil function (Chap. 7). Additionally, and importantly, we have developed a more biochemical understanding of process dynamics as shown in Chap. 8. Processes whose basic controls were recognized qualitatively in Waksman (1932) can now be described quantitatively, allowing us to develop simulation models of soil processes over large scales of space and time (Chap. 16).

While the last 70 years of the 20th century have thus been a period of immense growth and accomplishment in soil microbiology, biochemistry, and ecology, one can argue that much of the work during this period has largely been developing the full promise of the ideas and directions that were developed during the early part of the 20th century. We know vastly more about soil systems, of the diversity of soil organisms, and of the linkages between soil systems and ecosystems. However, "the information available (linking populations and processes) is far from satisfactory in explaining what is taking place in the soil" remains a true statement. Thus, this raises the question "What do we need to do to ensure that statement is no longer true when the next-generation textbook in this field is written in some years?"

Science advances through a feedback cycle in which theory develops in step with the tools to test and further develop those theories. Tools may limit the questions we can answer, but theory controls the questions we ask. Through much of the 20th century, technological developments were evolutionary, rather than revolutionary, and the theoretical advances paralleled them. The most obvious technical constraint that limited development was the inability to study microbial processes at the site of the activity and the inability to study the organisms without culturing them. The inability to study organisms and their processes at the spatial scale at which they actually live and interact still limits our research today. Measurements and thinking were based on aggregating at a scale a million times larger than a single organism. Measuring respiration from a soil core can be analogized to measuring the integrated $CO_2$ flux from North America. That is actually a useful thing to do (Dilling *et al.*, 2003), but lumping Death Valley and North

Dakota, and both heterotrophs and autotrophs as well as Archaea and nematodes, makes it impossible to evaluate who the important players are or how they function. Increasingly, in soil science, we are growing beyond treating "microsites" as a euphemism for "we can't explain it based on overall properties" and developing theory that treats the soil as a complex landscape (e.g., Schimel and Bennett, 2004; Allison, 2005).

Several technologies are allowing us to begin resolving the scale issues. Isotope tracers allow us to analyze chemical pathways and to evaluate gross rates of individual processes, rather than just net production of final products (Chap. 3). Microscale sensors will allow us to measure the environment and processes occurring at the microsite scale (Noll *et al.*, 2005). Microscopy and visualization tools (visual, confocal, electron, atomic force, etc.) are allowing us to examine soil organisms in their native habitat at the scale at which they actually live (Chap. 4). Finally, a key tool in bridging scales is simulation modeling (Chap. 16). The development of computing has been as necessary as the analytical tools themselves in allowing us to integrate knowledge from different approaches. The important question as to why models based on abiotic controls and soil organic matter pools and fluxes appear to work so well without more than an implicit interpretation of microbial population still has to be answered. It could be that we are not yet asking the most important questions and demanding exact enough answers.

Each of the new technologies, by itself, has produced exciting new science, as described in previous chapters. However, it is the ability to use new tools in synchrony that will power the soil biology of the 21st century. The theme for soil microbiology, ecology, and biochemistry over the next decades is likely to be "integration." Integrating knowledge of community composition and function, integrating information from multiple disciplines to better understand soil systems, and integrating across scales to better understand how processes that occur at micrometers play out at meters and kilometers.

Several areas are particularly ripe for effective integration to produce new, useful knowledge about the diversity and functioning of soil communities. The first of these is in linking biotic populations and the processes they carry out. This has been an important research area in macroecology (Hobbie, 1992) and with the advent of molecular methods for analyzing the microbial community *in situ*, we are finally able to address the challenge posed by Selman Waksman in 1926. When the techniques were becoming available for analyzing microbial community composition, the first papers published discussing the linkage between community composition and function tended to be theoretical, hypothesizing about when such linkages would be observable and using the limited case studies available to develop theory (e.g., Schimel, 1995; Groffman and Bohlen, 1999). Increasingly, however, research is using an integration of physiological (Chap. 3) and molecular tools (Chap. 4) to effectively link community composition and function (e.g., Zak *et al.*, 2003; Gallo *et al.*, 2004; Balser and Firestone, 2005; Smithwick *et al.*, 2005). By linking isotopic and molecular analyses we can evaluate which organisms are using which substrates and how fast. This can be done through both $^{13}C$ PLFA analyses (McMahon *et al.*,

2005) and stable isotope probing (Friedrich, 2006). Genomic and proteomic approaches are allowing us to evaluate which organisms have which genes and which are expressed *in situ* (Riesenfeld *et al.*, 2004; Schulze, 2004). Tying these to the basic understanding of physiology and biochemistry of soil organisms as discussed in Chap. 9 will allow us to truly determine the controls on important soil processes. This in turn will lead to the possibility of better managing human-affected systems in fields such as global change and soil and ecosystem sustainability.

Studies linking community composition and function raise several issues that provide direction for future research. One obvious question, as discussed in Chap. 8, is why are soil communities different? Although there is extensive work on characterizing soil communities, we have only limited and qualitative understanding of the factors that regulate community composition. The second issue is the challenge of scaling population–process linkages up to the ecosystem and even landscape level. A third issue is that microbial ecology has gone a somewhat separate path from that of macroecology. Both fields could benefit from more dialogue between them.

## SOIL COMMUNITY ECOLOGY—CONTROLS OVER POPULATION AND COMMUNITY DYNAMICS

At the broadest range of scales, soil communities vary among ecosystems in somewhat predictable ways. For example, coniferous forest soils tend to be dominated by fungi because only fungi have the enzyme systems to effectively break down wood (Chap. 12). Disturbed systems and some grasslands tend to be dominated by bacteria (Chap. 5). At much finer phylogenetic scales, species shifts within a plant functional group do not appear to cause substantial variation in the overall composition of the soil community (Porazinska *et al.*, 2003), although individual plant species can select for specific soil organisms (Bergsma-Vlami *et al.*, 2005). Other examples include plant–microbial interactions such as the mycorrhizas that may or may not be beneficial to the host plants depending on the specific soil-nutrient characteristics (Chap. 10). At more intermediate scales, the transition in growth form from shortgrass to tallgrass prairie does seem to cause shifts in microbial community composition (McCulley and Burke, 2004).

Environmental factors, such as drought, wetting–drying, and seasonal change, can cause substantial changes in the microbial community (Schimel *et al.*, 2006; Chap. 8). For example, in the Colorado alpine, there is a major shift between distinct summer and winter communities (Schadt *et al.*, 2003). The summer community is dominated by bacteria, while the winter community is more heavily fungal, with several previously unknown lineages of fungi dominating. The winter community is better at using phenolic compounds (Lipson *et al.*, 2002), and as it dies away in the spring the nutrient flush released provides the plant community with most of their nutrients to support summertime growth. This seasonal swing in the microbial

community thus dominates the annual nutrient cycle of the entire ecosystem (Jaeger *et al.*, 1999). However, the question remains unanswered—what specific factors cause the switch between communities? This example merely highlights the questions that remain to be answered and identify microbial community and population biology as fruitful areas of research. An interesting question in this arena is, how well will results and theories about community dynamics that have been developed in plant ecology apply in microbial ecology?

## MICROBIAL LIFE AT THE MICROBIAL SCALE—THE MICROBIAL LANDSCAPE

As we learn more about the factors that regulate microbial populations and activities, the more we realize that the soil is not a homogeneous medium. Rather, the opposite; it is the most physically complex environment for life on the planet. Soil is formed into complex aggregate and pore structures. Consumers, ranging from amoebae to arthropods, patrol the larger pores as they forage (Chap. 7). Soil biota likely cover less than 1% of the surface area of the soil particles, they may be trapped in soil micropores and they commonly live embedded in polysaccharide biofilms (Chap. 11).

This leads to the issue that while we often discuss "the" soil community, that language may be misleading. The soil community is likely not a single aggregate entity with thousands of species, but rather a spatially structured collection of many subcommunities, each of which may be quite distinct (Zhou *et al.*, 2002). The diversity we observe at the soil sample level may actually be gamma diversity—landscape-scale diversity among communities from the perspective of soil microorganisms—rather than alpha diversity—species diversity within a single area (Odum and Barrett, 2005). Increasingly, visualization and microscale analysis techniques are allowing us to observe and analyze soils at the spatial scales at which soil organisms actually live and will provide a better understanding of these dynamics.

The importance of viewing the soil as a heterogeneous biotic landscape is that each of the different sites that occur in soil, with different organisms and different physical/chemical conditions, may behave differently. The functioning of the overall soil system is not a simple average of different microsites (Schimel and Bennett, 2004). One well-known example of this is the rhizosphere, which is different from bulk soil (Chaps. 10 and 11). The flow of C, consumption of $O_2$, and release of specific C compounds produce an environment in which soil communities are distinct in terms of their composition, metabolic rates, and nutrient turnover characteristics (Chap. 10). This has been known for many years, yet few studies have analyzed or modeled the spatial extent and structure of rhizospheres and how much of total soil processes they account for. Adequately understanding the total system-level impact of roots and rhizospheres on the soil biota requires

integrating modeling of root architecture with models of root effects on organisms and soil food webs. This remains a challenge but is becoming more practicable.

Another well-known example of macroscale effects resulting from microscale structure is denitrification. This is an important process in soil and global N cycling and is an important influence on atmospheric chemistry through the production of $N_2O$ (Chap. 13). Denitrification occurs in anaerobic microsites in the soil and it is thought that these microsites can occur either in the interior of aggregates, where limited $O_2$ diffusion allows anaerobiosis (Sexstone *et al.*, 1985), or in organic matter-rich spots, where rapid $O_2$ consumption produces anaerobiosis (Parkin, 1987). As with the rhizosphere, developing a full picture of denitrification requires understanding the production and distribution of anaerobic microsites (an issue of soil structure and physics) with the distribution of denitrifying organisms in a three-dimensional modeling structure to evaluate the movement of $O_2$, $NO_3^-$, and bioavailable C within the soil. Such a concept is not new (Smith, 1980), but little recent work has been done in this area.

While denitrification has long been known to be largely a result of microsite dynamics, other N cycle processes appear to be sensitive to such phenomena as well. Nitrate is commonly taken up by soil microorganisms, even when substantial concentrations of $NH_4^+$ are measurable in the soil (e.g., Jackson *et al.*, 1989). This occurs despite physiological studies showing that in the presence of $NH_4^+$, microorganisms shut down $NO_3^-$ assimilation (Rice and Tiedje, 1989). How then, do you explain whole-soil $NO_3^-$ assimilation without postulating that there are sites where $NH_4^+$ is absent and microbes are N-limited and therefore can use $NO_3^-$? Another example is that plants take up N *in situ*, even in situations in which net mineralization assays (Chap. 13) measure net immobilization (Giblin *et al.*, 1991). Clearly, N is available and plant roots are able to intercept some portion of it when they are present. Schimel and Bennett (2004) proposed a microsite-based model of soil N cycling that integrates root interception of N moving between N-rich and N-poor microsites to explain changing patterns of plant N uptake across nutrient availability gradients.

Integrating a stronger spatial component into our understanding of soil processes is likely to require more effective incorporation of the role of consumers and pathogens in regulating microbial population dynamics. Different groups of fauna prefer different species of microorganisms (Ronn *et al.*, 2002), thus potentially regulating the dynamics of specific microbial populations and the processes they carry out. Selective feeding interacts with soil spatial structure because, although bacteria and fungi are largely immobile, consumers must be able to forage. Because microorganisms acquire resources via diffusion (enzymes away from and substrates back to the cell), they are able to exist in micropores and to remain active under dry conditions when water films are thin and microsites are hydrologically disconnected. Consumers and pathogens (e.g., bacteriophage), however, must be able to move to find new prey or hosts. For protozoa, viruses, and nematodes, this requires water-filled pores of appropriate size. Larger consumers, such as mites, require air-filled pores. The variation in pore structure and which organisms are able to function in each class of pores led to the development

of the “habitable pore space hypothesis” (Elliott *et al.*, 1980; Chap. 7). Physically structured, individual-based models of consumer–resource interactions have become common tools in macroecology (Murdoch *et al.*, 2003) and may well be necessary to fully integrate soil food webs and soil community dynamics.

Enhancing our understanding of spatial ecology is an important direction for developing soil biology and ecology. This flows naturally out of a focus on understanding soil community composition–function relationships. Microsite dynamics are important in regulating large-scale phenomena that have important influences on plant nutrition and other environmental processes. However, we still have limited understanding of how physical and biotic factors interact to produce microsites (Chap. 11). Developing a better understanding of how organisms are distributed relative to physical and environmental structures and gradients through the three-dimensional matrix of the soil will be an important objective in fully understanding soil diversity, the ecology of soil organisms, and the functioning of the soil system (Young and Crawford, 2004). Such research will require combining microscale analysis techniques, visualization, and *in situ* community analysis techniques (Chap. 4).

## A WHOLE PROFILE PERSPECTIVE

The first step up in scale from the individual soil sample is to the whole soil profile. The very definition of soil, according to the Soil Science Society of America (http://www.soils.org/sssagloss/), explicitly includes the entire profile that “has been subjected to and shows effects of genetic and environmental factors.” While organisms in surface soil are more active per gram soil, the vast mass of the deeper horizons adds up to a lot of potential activity (Richter and Markewitz, 1995). There are strong gradients of resource availability and environmental stress through the soil profile (Chap. 11), and these gradients control the composition and function of the soil community. Surface soil is substrate-rich from litter and root inputs, but experiences wide swings of temperature; it dries and wets regularly, and it is physically disturbed by animals (e.g., worms, gophers, and humans). However, deeper in the profile, substrate quantity decreases, but the environment becomes less variable. Soil communities through the profile are likely structured by these counterbalancing gradients of resources and stress (Wagener and Schimel, 1998; Zhou *et al.*, 2002). The best known theories regarding how stress and resources govern communities are from macroecology and concern intermediate disturbance and resource supply (Connell, 1978; Hobbie *et al.*, 1994; Tilman, 1994; Chap. 8). Biodiversity often (though not always) shows a maximum under moderate levels of resource or disturbance (Mittelbach *et al.*, 2001). Studies using gradients for relating population and community dynamics to environmental drivers have been powerful tools in macroecology (e.g., Connell, 1978; Tilman, 1984) and they should be equally powerful for understanding soil community and population dynamics.

## SCALING TO THE ECOSYSTEM

Scaling to the whole profile represents only the first dimension in scaling our understanding of soil processes up to the level of the whole ecosystem. While important basic science can be done at the micro- and mesocosm levels, application of the knowledge gained at those scales requires relating it to the scales at which human action and policy are carried out. Traditionally, "scaling" has often meant either doing work at small scales and then extrapolating it up or doing work at large scales and interpolating it down. Both approaches have limitations—extrapolating risks up misses emergent phenomena (Chap. 8), while interpolation requires assumptions about homogeneity. Effectively scaling knowledge requires designing experiments that are directly targeted at bridging scales, simultaneously collecting compatible and linked data sets at both micro- and ecosystem scales. The second key tool for integrating knowledge from multiple scales is modeling, assimilating the information from each scale into an integrated package (Chap. 16). While soil community modeling is not well developed, it is going to be a necessary tool for developing a better understanding of soil organism dynamics as they play out at the ecosystem level. One approach to modeling will be to take small-scale understanding and link it up into spatially complex structures. The other approach will be to take models structured at larger scales and compare their outputs to complex data sets to test different sets of assumptions about microbial behavior.

The current generation of ecosystem models includes soil biology "implicitly," by incorporating knowledge of biotic dynamics in response functions and parameter sets, rather than by incorporating the soil community explicitly (Schimel, 2001). Ecosystem models generally make assumptions about the microbiota that may be valid under equilibrium conditions (Schimel, 2001), but increasingly modeling is focusing on common nonequilibrium conditions, such as seasonal transitions, plant invasions and other community changes, climate change, and pulse disturbances such as drying–rewetting and freeze–thaw (Yuste *et al.*, 2005). Early work is suggesting that to capture such pulse events adequately, models must consider the functional dynamics of the soil community—die-offs and shifts in functional characteristics (Li *et al.*, 2006). This may require new approaches to modeling processes that somehow incorporate soil communities as the link between changes in the environment and changes in processes. This flow involves several steps (Fig. 18.1).

First, in the long term, environmental factors (including microclimate, soil texture, substrate availability, etc.) regulate the composition and diversity of the soil community. Over a shorter time scale, the same environmental factors acting on the organisms present regulate their physiological state—are they active, stressed, etc.? Finally, the organisms present and their physiological state directly regulate the processes that they actually carry out. Developing a complete understanding of environmental control over soil process dynamics may require understanding the complete flow of control from environment through community composition and physiological state through to process dynamics. Once that understanding is

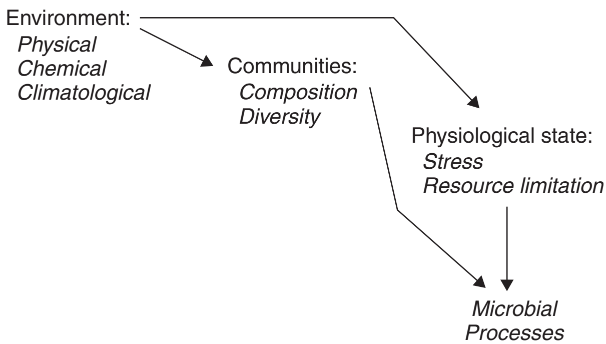



FIGURE 18.1 Linkages between environmental factors, microbial community composition, physiological state, and process dynamics.

developed it should become possible to simplify and condense the information to develop models that go directly from environmental factors to response functions and process rates, but in a more mechanistic way that captures the essential organismal dynamics and is more robust to a changing environment than current models. Developing this level of understanding will require highly integrated, interscale study of the physical, chemical, and biological processes that occur in soil.

## APPLICATION

Ultimately better understanding of population–process linkages, the spatial ecology of soil organisms, and how to scale this knowledge to the ecosystem should provide increased power for applying this knowledge. How do we manipulate soil systems to achieve desired goals—increased nutrient cycling efficiency, reduced greenhouse gas emissions, pollutant biodegradation, C sequestration, and invasive plant species control (Chap. 17). The 21st century has been described as the "Biotech Century" (Rifkin, 1998) We are already learning that in environmental applications, ignoring the ecology of the soil biota is a likely path to failure. For example, in the early days of genetically engineered microorganisms, there was a lot of concern about environmental release (Tiedje *et al.*, 1993). Most studies, however, found the biggest problem was getting the organisms to survive long enough to do what you want them to! In some cases, adding organisms is effectively just adding the nutrients contained within them that are released when they die. Thus, it has become clear that effective environmental applications of biotechnology, whether using introduced or existing organisms, require a solid understanding of the organisms' ecology (Chaps. 8 and 17).

One target for applied soil biology is pollutant management. Microorganisms have diverse physiological capabilities that allow them to degrade a wide range of organic chemicals (Chap. 17). Thus, by either applying microbes to a contaminated

soil or, more likely, stimulating the activity of organisms already present, contaminants may be able to be degraded *in situ*, with relatively limited site disturbance and at modest cost.

Soil microbiology, ecology, and biochemistry are also likely to play increasingly important roles in agriculture. Using legume crops for N fixation has been part of many agricultural systems over the millennia (Chap. 14). This is certain to continue and be especially relevant as we develop energy crops for alternatives to fossil fuels. Biological N fixation can offset the high energy costs of fertilizer N. It also should have the potential of fixing N only when needed, thus reducing environmental pollution by unused fertilizer N. An important question that must be addressed is whether it will be viable to introduce N fixation into high-energy crops such as corn and switch grass. It already is present to some extent in some sugarcane production. The alternative that should be explored is whether currently efficient N-fixing symbiotic systems could be genetically altered to become high-energy crops. Researchers have been trying to take advantage of mycorrhizas to enhance plant nutrient uptake and productivity (Chap. 10), though this has seen limited success so far and will always raise the challenge that by enhancing plant uptake of soil resources, you also deplete the soil of those same resources—they must be replaced or the soil will inevitably degrade. This idea of managing ecosystems by managing their soil biota may also be applied to other ecosystem types to manage for environmental processes like C sequestration and trace gas emissions as well. Whether we can manage soil communities to manage the global environment better will depend on learning how to manipulate these communities better in nature, rather than just genetically engineering organisms in the laboratory.

## CONCLUSIONS

In the early years of the 21st century, we are in a period of revolutionary change in soil microbiology, ecology, and biochemistry. New tools are allowing us to ask, and answer, questions about the nature and consequences of life in soil to an extent that was matched only by the initial development of microbiology at the end of the 19th century. Genetic tools allow us to characterize the composition of the community. Isotopes, proteomics, and other tools allow us to characterize the physiology of organisms *in situ.* Equally important to developing soil biology, however, are new ideas and a new theoretical framework that describes how the soil biota functions at its native scales and how those influences resonate at human scales. The next several decades will be a period of challenge, opportunity, and change.

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